```
Gly205Asn + Thr207Gln
Ser210Glu + Leu211Pro
Leu 94Gln + Ser210Glu
Ala209Asp + Leu211Pro
Asn 60Glu + Leu211His
Ala 96Asn + Thr214Gly
Asn 60Glu + Ala194Gly
Ser103Asp + Ala181His
Gly125Glu + Ala209Asn
Ser158Asp + Asn198Ser
Gly155Ser + Asn198Ser
Ser104Asp + Gly152Asn
Ser182Asp + Leu211Cys
Val 93Ser + Gly125Glu
Asn198Glu + Leu211Ser
Gly100Ser + Ser103Asp
Ala181Gln + Gly213Gln
Thr 64Gly + Ser126Asp
Ile159Cys + Gln200Asp
Thr207Glu + Leu211Asn
Ser104Glu + Pro204Gly
Ser130Asp + Pro204Asn
Pro129Gln + Gln185Asn
Asn 60Glu + Gly157Ser
Gly125Pro + Val197Met
Asn 60Glu + Ile105Cys
Pro127Gln + Val197Thr
Ser101Asp + Leu211Ala
Gln 57Ser + Ser 99Glu
Phe183Pro + Asn198Glu
Asn198Glu + Thr202Pro
Val197Gln + Leu211His
Gly 61Ser + Gln200Ser
Gly 61Asp + Asn198Gln
Gly 61Glu + Phe183Ser
Thr207Asn + Asn212Glu
Tyr203Thr + Tyr208Gly
Asn198Ser + Leu211Cys
Asn153Asp + Leu211Ser
Gln 57Asn + Gly213Pro
Thr 64Ser + Ser210Asp
Pro204Asp + Leu211Ile
Gln 57Asp + Gly 61Pro
Gly155Gln + Ser210Asp
Ala156Gly + Thr207Asn
Gly 61Gln + Ala181Thr
Ile159Cys + Asn198Gln
Pro129Gly + Leu211Glu
Asn198Glu + Thr207Ser
Gly 98Ser + Ser128Asp
Ala131Asn + Val193Ser
Gln185Ser + Gln200Asn
Ser210Asp + Leu211His
Gly125Glu + Ala156Gln
```

```
       Ser  97Asp  +  Gly125Gln
       Asn  60Gln  +  Gly155Pro
       Ser126Glu  +  Thr207Ser
       Phe183Met  +  Thr207Pro
 5     Ser154Glu  +  Phe183Ile
       Val  93Gln  +  Ser210Asp
       Val  93Gln  +  Tyr208His
       Asn  60Ser  +  Asn198Glu
       Gly152Gln  +  Gln200Asp
10     Pro127Asn  +  Gly152Asn
       Gly155Gln  +  Thr214Asn
       Ala  96Ser  +  Tyr208His
       Tyr161Thr  +  Ser210Asp
       Gly100Pro  +  Pro204Gln
15     Gln  57Asp  +  Ala194Asn
       Asn  60Gln  +  Thr202Asn
       Gly157Pro  +  Gly213Asn
       Val  93Thr  +  Leu211Ala
       Ser103Glu  +  Leu211Thr
20     Ser101Asp  +  Leu124Ile
       Leu  94Glu  +  Pro204Gln
       Gln200Glu  +  Ala209His
       Ser101Glu  +  Gly152Gln
       Asn198Asp  +  Gly205Pro
25     Gly  61Ser  +  Gln200Asn
       Ser158Asp  +  Val197Asn
       Gly  61Glu  +  Phe183Ile
       Asp  58Glu  +  Tyr203Ser
       Gly213Ser  +  Thr214Gly
30     Gln  57Asp  +  Asn198Gln
       Tyr102Leu  +  Ser158Glu
       Ala  96Gln  +  Leu211Asn
       Val  93Asp  +  Thr202Asn
       Leu  94Ser  +  Asn198Asp
35     Gly196Ser  +  Asn212Asp
       Gly155Asn  +  Gly157Asp
       Val  93Asn  +  Ser130Asp
       Leu  94Ile  +  Ser210Asp
       Val197Ala  +  Ser210Asp
40     Ser104Asp  +  Gly205Pro
       Asn153Asp  +  Ala181Gly
       Gln200Asn  +  Leu211Glu
       Leu  94Asn  +  Ser210Glu
       Gln185Asp  +  Val197Gln
45     Tyr102Ser  +  Thr207Asp
       Gly  61Glu  +  Gly  95Asn
       Gly  61Asp  +  Pro129Gln
       Ser  99Asp  +  Thr207Ser
       Ser126Glu  +  Gly152Gln
50     Val197His  +  Ser206Glu
       Gly  61Asn  +  Gly155Glu
       Gly155Asn  +  Asn198Glu
       Ala209Asn  +  Ser210Glu
       Ser126Asp  +  Thr207Gly
```

Ala209Thr + Ser210Asp
Gln185Asn + Gly213Pro
Ser126Glu + Leu211Gly
Ser 97Asp + Gly205Ser
Leu 94Thr + Leu124Thr
Asn 60Gln + Leu211Gly
Pro129Asp + Gly155Pro
Asp 58Glu + Gly157Ser
Thr202Pro + Thr207Glu
Ala156Ser + Tyr208Asp
Gly 98Glu + Tyr161Gln
Ile105Cys + Gln200Asn
Gln200Glu + Pro204Gly
Gly 98Pro + Ala131Asn
Gly 59Asp + Thr207Gln
Tyr102Gln + Ala209Gln
Gly 98Glu + Ile105His
Ser101Asp + Pro129Gln
Pro127Asp + Gly213Asn
Ser104Glu + Asn198Gln
Gln 57Ser + Gln200Asn
Val197Cys + Gln200Ser
Val 93Ala + Ala156Glu
Gln200Asn + Gly205Glu
Leu 94Ala + Thr207Asn
Ala131Thr + Gln200Ser
Leu 94Glu + Tyr161Thr
Gln200Asn + Ser210Asp
Gln 57Asp + Thr207Asn
Ser 99Glu + Ala156Pro
Leu124Ile + Asn198Ser
Tyr161Gly + Thr207Gln
Ile105Cys + Thr214Asp
Ile159His + Ser206Asp
Gly 63Gln + Gly125Asn
Asn198Asp + Gly205Ser
Ser104Glu + Leu124Gln
Ala209Pro + Ser210Glu
Tyr161Val + Asn198Asp
Pro129Ser + Gln200Asp
Ser 97Asp + Tyr203Ser
Ser103Asp + Phe183Thr
Tyr161Gly + Val197Ala
Asn198Gln + Pro204Glu
Leu211Gly + Asn212Glu
Ser206Glu + Leu211Thr
Gly152Pro + Val197Ser
Ile105Ala + Ala156Gln
Gly 63Asn + Tyr102Pro
Ser182Glu + Val197Asn
Thr 64Glu + Ala 96Gln
Asn 60Gln + Ser206Glu
Asn 60Gln + Ser128Asp
Ser101Asp + Ile159Pro

```
         Gly100Ser + Thr207Ser
         Gly 63Asn + Gly205Ser
         Ala181Glu + Thr207Asn
         Gly125Ser + Tyr161Ser
    5    Gln200Glu + Leu211Ala
         Ser101Asp + Tyr102Leu
         Ser126Glu + Thr207Pro
         Gly 59Asp + Tyr102Thr
         Gly100Gln + Tyr102Met
   10    Gly 59Ser + Asn198Asp
         Asn 60Ser + Leu 94Cys
         Pro127Glu + Gly205Pro
         Gln 57Glu + Ala156Asn
         Ala131Asn + Thr207Asp
   15    Ala131Asp + Thr202Ser
         Leu124His + Ala131Asn
         Gly152Glu + Leu211His
         Ser101Glu + Ala181Thr
         Asp 58Glu + Gly205Ser
   20    Gly 61Asn + Gln200Asp
         Pro129Gly + Val197Asp
         Thr 64Gln + Asn198Ser
         Val 93Ala + Ser154Glu
         Ser130Glu + Tyr161Val
   25    Phe183Asp + Leu211Thr
         Ser206Glu + Asn212Gln
         Gly 63Ser + Ala131Glu
         Pro127Asn + Gln200Asp
         Ser130Asp + Phe183Thr
   30    Ala181Thr + Pro195Ser
         Gly 95Ser + Ala156Glu
         Tyr161Gln + Tyr208Ile
         Gln 57Asp + Leu211Cys
         Asn153Gln + Gly213Asn
   35    Val 93Thr + Leu124Ser
         Ser101Glu + Asn212Gln
         Gly157Ser + Asn198Glu
         Gly152Ser + Ser182Glu
         Tyr161Gln + Gly213Ser
   40    Pro195Gly + Gln200Asn
         Asn 60Asp + Leu211Pro
         Pro195Ser + Tyr208Glu
         Gln200Asp + Leu211Asn
         Leu124Val + Thr207Pro
   45    Thr 64Pro + Ser126Glu
         Thr 64Ser + Tyr203Val
         Ser 97Glu + Asn198Ser
         Gly152Gln + Leu211Pro
         Asn198Asp + Leu211Asn
   50    Val 93Glu + Gly125Gln
         Gly 98Asn + Gln200Asp
         Gln200Asn + Thr207Asp
         Asn198Ser + Gln200Glu
         Tyr102Leu + Asn198Gln
```

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```
Leu124Cys + Asn153Ser
Asn198Asp + Leu211Pro
Ser126Glu + Tyr208Met
Ala  96Asn + Gly100Ser
Ser  99Glu + Leu211Cys
Ser  97Asp + Leu211Ala
Ser182Glu + Leu211His
Ile159Cys + Leu211Glu
Gly152Asn + Ser210Asp
Val197Ala + Gln200Glu
Asn  60Ser + Ser158Glu
Ser104Glu + Tyr208Asn
Pro204Asp + Leu211Gly
Asp  58Glu + Val197Gly
Ser182Glu + Tyr208Ser
Ser103Asp + Ala194Ser
Ala156Gln + Thr207Asp
Asn198Ser + Thr207Ser
Gln  57Asp + Thr207Ser
Gly152Glu + Thr207Gly
Ala131Ser + Ile159Asn
Pro127Asn + Gly152Glu
Gln200Asn + Gly213Glu
Leu124Asp + Leu211Ala
Gly  98Glu + Gly125Asn
Ile159Gly + Thr207Asn
Pro127Gln + Asn153Asp
Ser103Glu + Phe183Tyr
Ser160Glu + Pro204Ser
Gly157Ser + Tyr161His
Ala156Pro + Ser158Glu
Gly100Glu + Pro127Asn
Gln  57Asn + Asn  60Glu
Gly125Ser + Ser210Glu
Gly125Pro + Ser128Asp
Gly125Glu + Val197Gly
Val193Met + Asn198Ser
Ser160Glu + Gly213Asn
Ser206Glu + Ala209His
Ser  97Glu + Gly213Ser
Gly100Asp + Leu211Gly
Pro129Gln + Thr207Gly
Gly  95Gln + Ser210Asp
Gln185Ser + Pro204Glu
Pro129Glu + Ala156Ser
Ser126Asp + Asn212Ser
Gly  59Ser + Ile159Ala
Ala  96Asn + Ser  99Glu
Ser104Asp + Ile159Val
Gly  63Asn + Ser210Glu
Phe183Pro + Ser210Glu
Ala  96Asp + Tyr208Ile
Gly  98Asp + Thr207Gln
Val  93Gln + Gly125Glu
```

```
      Ile159Glu + Thr207Gln
      Leu 94Ile + Leu211Val
      Gly157Asp + Leu211His
      Gly 61Asp + Leu211Cys
      Gln 57Asn + Gly157Asn
      Gly100Glu + Val197Ser
      Gln200Glu + Pro204Asn
      Ala131Gly + Leu211His
      Ser206Asp + Leu211Asn
      Asn153Asp + Ile159Leu
      Gly152Ser + Asn198Glu
      Leu 94Met + Ser130Glu
      Gln 57Glu + Asn198Gln
      Ser182Glu + Leu211Thr
      Val199Ala + Ser210Glu
      Thr207Gly + Ser210Glu
      Thr 64Gly + Gly 98Asp
      Gly 61Gln + Ser 99Asp
      Gly155Glu + Leu211Ser
      Leu124Ser + Thr207Asp
      Val 93Ser + Asn198Gln
      Ser 99Asp + Gly125Asn
      Gln 57Asn + Asn198Glu
      Ser 99Glu + Ile159Met
```

TABLE 34

Multi-loop Triple Mutation Variants
Gln 57Ser + Leu 94Gly + Gln200Glu
Asn 60Ser + Val 93Gln + Gly213Asp
Tyr102Cys + Asn198Gln + Thr207Gly
Leu124Ile + Ser154Glu + Asn198Gln
Leu 94Gly + Ala209Gly + Ser210Glu
Gly155Asp + Ala209Gln + Asn212Gln
Pro129Asn + Gly157Asn + Thr207Ser
Asn198Gln + Gln200Asp + Ala209Pro
Val 93Ala + Pro127Asp + Thr202Ser
Tyr161Asn + Thr207Asp + Leu211Met
Gly 61Ser + Pro127Asn + Thr207Pro
Gly 61Pro + Leu124Asp + Pro204Asn
Gln 57Glu + Gln185Ser + Asn198Ser
Gly 59Asn + Asn 60Glu + Gln200Ser
Gly 63Gln + Gln200Asp + Leu211Gly
Tyr203Ser + Thr207Gly + Gly213Glu
Gly 63Asn + Gln185Asp + Ala209Asn
Pro129Asn + Ser210Glu + Leu211Asn
Tyr102Ala + Gly125Glu + Leu211Gly
Gln 57Asn + Gly155Gln + Leu211His
Ile105Pro + Gly125Ser + Thr207Gly
Asn198Ser + Thr207Asn + Ser210Asp
Gly 59Gln + Gly 95Ser + Ala156Glu
Tyr102Cys + Ser210Glu + Leu211Asn
Gly205Asn + Thr207Gln + Ser210Glu

```
        Leu 94Gln + Ala209Asp + Leu211Pro
        Asn 60Glu + Leu211His + Thr214Gly
        Asn 60Glu + Ala181His + Ala194Gly
        Ser104Asp + Gly155Ser + Asn198Ser
   5    Gly152Asn + Ser182Asp + Leu211Cys
        Gly100Ser + Ser163Asp + Leu211Ser
        Thr 64Gly + Ser126Asp + Ile159Cys
        Ser130Asp + Ala181Asn + Pro204Gly
        Pro129Gln + Gly157Ser + Gln185Asn
  10    Asn 60Glu + Gly125Pro + Val197Met
        Ile105Cys + Pro127Gln + Val197Thr
        Gln 57Ser + Ser 99Glu + Phe183Pro
        Gly 61Ser + Val197Gln + Leu211His
        Gly 61Asp + Asn198Gln + Gln200Ser
  15    Tyr203Thr + Thr207Asn + Tyr208Gly
        Asn153Asp + Asn196Ser + Leu211Ser
        Gln 57Asn + Ser210Asp + Gly213Pro
        Thr 64Ser + Pro204Asp + Leu211Ile
        Ser101Asp + Ala156Gly + Thr207Asn
  20    Ile159Cys + Ala181Thr + Asn198Gln
        Gly 98Ser + Asn198Glu + Thr207Ser
        Ser128Asp + Ala131Asn + Val193Ser
        Gln185Ser + Gln200Asn + Leu211His
        Ser126Glu + Phe183Met + Thr207Ser
  25    Ser154Glu + Phe183Ile + Thr207Pro
        Val 93Gln + Tyr208His + Ser210Asp
        Val 93His + Pro127Asn + Gly152Gln
        Gly155Gln + Gly213Glu + Thr214Asn
        Gly100Pro + Tyr161Thr + Ser210Asp
  30    Gln 57Asp + Ala194Asn + Pro204Gln
        Gly157Pro + Thr202Asn + Gly213Asn
        Thr 64Gln + Val 93Thr + Leu211Ala
        Gly 61Ser + Gln200Asn + Gly205Pro
        Ser158Asp + Phe183Ile + Val197Asn
  35    Asn198Asp + Gly213Ser + Thr214Gly
        Gly152Ser + Gly155Ser + Ser206Asp
        Gly152Gln + Gln185Ser + Val197Gln
        Leu 94Ile + Val197Ala + Ser210Asp
        Ala181Gly + Gln200Asn + Leu211Glu
  40    Leu 94Asn + Val197Gln + Ser210Asp
        Gly 61Asp + Pro129Gln + Thr207Ser
        Gln 57Asn + Gly 61Asn + Gly155Glu
        Gly 61Pro + Ser128Asp + Ala209Thr
        Thr207Gly + Ala209Thr + Ser210Asp
  45    Ser128Asp + Gln185Asn + Gly213Pro
        Leu 94Thr + Leu124Thr + Gly205Ser
        Asn 60Gln + Gly155Pro + Leu211Gly
        Asp 58Glu + Gly157Ser + Thr202Pro
        Gly 98Glu + Ile105Cys + Gln200Asn
  50    Gly 98Pro + Gln200Glu + Pro204Gly
        Gly 59Asp + Ala131Asn + Thr207Asn
        Gly 98Glu + Tyr102Gln + Ala209Gln
        Ser101Asp + Ile105His + Pro129Gln
        Pro127Asp + Asn198Gln + Gly213Asn
```

```
           Ile159Gly + Val197Gly + Asn198Gln
           Gln 57Ser + Gly155Glu + Gln200Asn
           Val197Cys + Asn198Gly + Gln200Ser
           Leu 94Ala + Gln200Asn + Gly205Glu
     5     Ala131Thr + Gln200Ser + Thr207Asn
           Leu124Ile + Ala156Pro + Asn198Ser
           Val 93Glu + Tyr161Gly + Thr207Asn
           Gly 63Gln + Gly125Asn + Ala209Glu
           Tyr161Gly + Val197Ala + Asn196Gln
    10     Ser158Asp + Asn198Ser + Leu211Gly
           Ile105Ala + Gly152Pro + Val197Ser
           Gly 63Asn + Tyr102Pro + Ala156Gln
           Ala 96Gln + Ser182Glu + Val197Asn
           Asn 60Gln + Ser101Asp + Ile159Pro
    15     Gly100Ser + Gly205Ser + Thr207Asn
           Tyr161Ser + Ala181Glu + Thr207Asn
           Gly125Ser + Gln200Glu + Leu211Ala
           Gly 59Asp + Tyr102Thr + Thr207Pro
           Gly 59Ser + Gly100Gln + Tyr102Met
    20     Asn 60Ser + Leu 94Cys + Asn198Asp
           Pro127Glu + Ala156Asn + Gly205Pro
           Asp 58Glu + Ala181Thr + Gly205Ser
           Thr 64Gln + Val 93Ala + Asn198Ser
           Phe183Asp + Leu211Thr + Asn212Gln
    25     Pro127Asn + Ser130Asp + Phe163Thr
           Tyr161Gln + Tyr208Ile + Leu211Cys
           Leu124Ser + Asn153Gln + Gly213Asn
           Val 93Thr + Ser101Glu + Asn212Gln
           Tyr161Gln + Gln200Asn + Gly213Ser
    30     Gln200Asp + Thr207Pro + Leu211Asn
           Thr 64Pro + Leu124Val + Ser126Glu
           Thr 64Ser + Ser 97Glu + Asn198Ser
           Gly152Gln + Ser210Asp + Leu211Pro
           Val 93Pro + Asn198Ser + Gln200Glu
    35     Thr 64Gln + Tyr102Leu + Asn153Ser
           Ala 96Asn + Gly100Ser + Tyr208Asn
           Ile159Cys + Ser182Glu + Leu211His
           Asn 60Ser + Ser158Glu + Tyr208Asn
           Ser103Asp + Ala194Ser + Tyr208Ser
    40     Ala156Gln + Asn198Ser + Thr207Asp
           Ala131Ser + Gly152Glu + Ile159Asn
           Pro127Asn + Gln200Asn + Gly213Glu
           Gly 98Glu + Gly125Asn + Leu211Ala
           Ile159Gly + Thr207Asn + Ser210Glu
    45     Ser103Glu + Phe183Tyr + Pro204Ser
           Gly157Ser + Ser160Glu + Tyr161His
           Gln 57Asn + Gly100Glu + Pro127Asn
           Gly125Pro + Val197Gly + Ser210Glu
           Val193Met + Asn198Ser + Gly213Asn
    50     Gly 95Gln + Pro129Gln + Thr207Gly
           Gly 59Ser + Ser126Asp + Asn212Ser
           Gly 63Asn + Ile159Val + Ser210Glu
           Phe183Pro + Tyr208Ile + Ser210Glu
           Leu 94Ile + Ile159Glu + Thr207Gln
```

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```
Gln  57Asn  +  Gly157Asn  +  Leu211Cys
Ala131Gly  +  Pro204Asn  +  Leu211His
Ile159Leu  +  Ser206Asp  +  Leu211Asn
Leu  94Met  +  Gly152Ser  +  Asn198Glu
Ser182Glu  +  Val199Ala  +  Leu211Thr
Leu124Ser  +  Thr207Asp  +  Leu211Ser
Val  93Ser  +  Gly125Asn  +  Asn198Gln
Tyr102Pro  +  Ala156Ser  +  Phe183Asn
Gly  98Gln  +  Ser128Glu  +  Ile159Cys
Ser104Asp  +  Ile105Pro  +  Tyr161Thr
Tyr102Pro  +  Ile105Thr  +  Ala181Glu
Thr  64Ser  +  Ile105Glu  +  Gln200Asn
Ser104Asp  +  Gln200Ser  +  Thr214Pro
Thr  64Pro  +  Ala156Glu  +  Thr202Gly
Gly  63Gln  +  Gly100Asp  +  Leu211His
Asn  60Ser  +  Gly  98Asp  +  Leu211Thr
Tyr161Gly  +  Val197Cys  +  Ser206Glu
Thr207Gln  +  Tyr208Gln  +  Ser210Glu
Gly  98Ser  +  Ile105Leu  +  Ser210Glu
Thr  64Gly  +  Pro195Ser  +  Thr214Ser
Val  93Met  +  Ser154Asp  +  Leu211Met
Leu124Ser  +  Asn198Ser  +  Leu211Asp
Thr  64Gly  +  Ala156Ser  +  Gly157Pro
Gly  59Ser  +  Gln200Asn  +  Gly213Glu
Gly  61Asn  +  Leu124Ile  +  Ala181Pro
Ala  96His  +  Gln185Asp  +  Leu211Ala
Ser101Asp  +  Asn198Ser  +  Thr214Ser
Leu  94Met  +  Asn153Asp  +  Pro195Gln
Pro127Gln  +  Gly152Asp  +  Val193His
Ser  97Asp  +  Ile105Thr  +  Thr207Gln
Gly  61Pro  +  Ser160Glu  +  Thr207Gln
Leu124Val  +  Pro204Gly  +  Leu211Ile
Ser  99Asp  +  Asn198Gln  +  Gln200Ser
Ser101Glu  +  Ala181Asn  +  Ala209Pro
Gly  61Asn  +  Ala  96Thr  +  Gly  98Ser
Val  93Pro  +  Asn198Asp  +  Thr207Gln
Thr  64Pro  +  Ala  96Pro  +  Gly155Glu
Thr  64Gly  +  Ala181Thr  +  Thr214Pro
Thr  64Pro  +  Leu  94Cys  +  Tyr208Met
Gln  57Asn  +  Leu  94Gln  +  Thr214Asn
Leu  94Gln  +  Tyr161Pro  +  Thr207Gly
Val  93His  +  Gln185Asn  +  Thr207Pro
Gly125Pro  +  Asn198Glu  +  Gln200Ser
Asn153Ser  +  Ile159Cys  +  Ser160Asp
Thr  64Gly  +  Gln200Glu  +  Leu211Cys
Leu  94Val  +  Pro195Gln  +  Leu211Asp
Gly  59Pro  +  Gly100Ser  +  Thr207Pro
Tyr102Cys  +  Gly157Gln  +  Thr207Pro
Ser  99Asp  +  Leu124Ser  +  Thr214Asn
Gly  59Gln  +  Gln200Asp  +  Thr207Ser
Ala  96His  +  Ala181Asp  +  Leu211Cys
Val  93Met  +  Ala181His  +  Thr207Ser
Pro129Gln  +  Asn198Ser  +  Thr207Ser
Asp  58Glu  +  Gly  95Ser  +  Ala194Gly
```

```
          Ser160Glu + Thr207Gln + Thr214Gly
          Thr 64Asn + Phe183Asn + Thr207Asn
          Ser158Asp + Phe183Ile + Val197His
          Gly 95Pro + Asn153Ser + Gln200Glu
     5    Gly157Asp + Pro204Gly + Thr207Ser
          Val197Pro + Asn198Gln + Thr207Gln
          Gly 61Pro + Leu 94Glu + Tyr161Ile
          Pro129Gln + Gly155Pro + Ser210Glu
          Val 93Met + Ser182Asp + Gln200Ser
     10   Pro127Ser + Leu211Met + Gly213Ser
          Ala 96His + Ser160Asp + Tyr208Gly
          Gly 98Pro + Pro129Ser + Pro204Asp
          Gly 59Ser + Gly 63Pro + Pro129Asp
          Gly100Pro + Leu124Pro + Thr207Asn
     15   Ser103Asp + Ile159Ala + Thr202Thr
          Gly 59Asn + Ser130Glu + Leu211Thr
          Ala 96Gly + Ala131Asn + Gly155Pro
          Asn153Ser + Gln200Asn + Ser210Glu
          Asn153Glu + Thr207Pro + Leu211Ala
     20   Pro127Asn + Val197His + Thr207Asp
          Gly100Asp + Ala131Gln + Tyr208Thr
          Gly125Pro + Phe183Pro + Asn198Glu
          Asn 60Ser + Ile105Thr + Thr207Glu
          Thr 64Ser + Pro127Asp + Ala131Thr
     25   Ser 99Asp + Leu124Asn + Phe183Pro
          Thr 64Gln + Ser101Asp + Leu211Ser
          Pro195Gln + Val197Gly + Leu211Ser
          Gly100Asn + Tyr102Gly + Thr207Pro
          Asn198Ser + Gln200Asp + Leu211Pro
     30   Tyr102Met + Ser182Glu + Leu211Ala
          Gly 61Asn + Gln200Asn + Gly213Asn
          Ser 99Asp + Gly152Pro + Gly157Ser
          Leu124Ser + Tyr161Met + Gln200Glu
          Tyr102Met + Leu211Glu + Thr214Ser
     35   Asp 58Glu + Gly 96Asn + Tyr161Asn
          Ile159Ser + Gln200Asn + Tyr208Asn
          Thr 64Asn + Ala156Gly + Leu211Asp
          Gln200Asn + Ser210Glu + Leu211Thr
          Asn 60Asp + Gly155Pro + Val197Asn
     40   Ala156Gln + Asn198Glu + Gly205Gln
          Gln 57Asp + Gln200Ser + Tyr208Thr
          Leu124Ile + Ala209Glu + Ser210Asp
          Gly 59Glu + Asn 60Asp + Leu211Ala
          Ala131Gly + Gly152Asp + Asn153Glu
     45   Tyr203Val + Ser210Glu + Leu211Glu
          Gly152Asn + Ser210Asp + Leu211Glu
          Phe183Val + Ser210Asp + Leu211Asp
          Tyr102Leu + Ser210Asp + Leu211Glu
          Gln200Asn + Thr207Glu + Tyr208Glu
     50   Ala156Ser + Thr207Glu + Tyr208Glu
          Pro129Asp + Ser130Asp + Asn198Ser
          Asn198Ser + Gln200Glu + Ser210Asp
          Leu124Cys + Gln200Glu + Ser210Glu
          Thr 64Gly + Gln200Asp + Ser210Asp
```

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```
Gly 61Asn + Gln200Asp + Ser210Glu
Phe183His + Val197Glu + Asn212Asp
Ala 96Gly + Ser101Glu + Ser103Glu
Ser126Glu + Ser128Glu + Asn198Ser
Gly 61Pro + Ser154Asp + Ala156Asp
Val 93Asp + Ser104Asp + Leu211Ala
Ala 96Ser + Gly125Asp + Tyr161Asp
Ser 97Asp + Ser 99Glu + Leu211Cys
Ser 97Asp + Ser 99Glu + Asn153Gln
Ala 96Pro + Gly152Asp + Ser154Asp
Gly 98Asn + Asn198Asp + Gln200Asp
Asn 60Ser + Asn198Glu + Gln200Asp
Pro129Ser + Asn198Asp + Gln200Asp
Gln 57Glu + Gly 59Glu + Leu211Gln
Ser128Asp + Ser130Glu + Ala209His
Ser158Asp + Ser160Glu + Gln185Glu
Pro127Gln + Ser210Glu + Asn212Asp
Ser210Glu + Leu211Asn + Asn212Asp
Val197Asp + Gln200Glu + Ser210Asp
Phe183Glu + Tyr208Val + Gly213Asp
Pro127Glu + Gly152Glu + Tyr161Asp
Leu 94Glu + Ser101Asp + Leu124Ile
Leu 94Glu + Ser101Asp + Gln200Asn
Pro127Gln + Asn153Glu + Ala156Glu
Asp 58Glu + Gly 61Gln + Gly 95Asp
Pro129Gly + Val197Asp + Ser210Glu
Asp 58Glu + Gly 61Glu + Gly 95Glu
Val197Glu + Ser210Asp + Gly213Asp
Ser154Glu + Ser182Glu + Pro204Ser
Ser101Glu + Gly125Glu + Ser126Glu
Ser 99Asp + Ser104Asp + Ile105Leu
Ser128Glu + Ala131Glu + Gly196Gln
Gly 61Glu + Ser 97Asp + Ala131His
Ser 97Asp + Gly100Asp + Ser104Glu
Asp 58Glu + Pro204Asn + Gly205Asp
Gln185Glu + Leu211Cys + Gly213Asp
Gln200Asp + Thr207Glu + Leu211Asn
Gly125Ser + Gln200Asp + Thr207Glu
Gln200Glu + Thr207Asp + Leu211Cys
Asp 58Glu + Val 93Gly + Ser 97Glu
Asp 58Glu + Ser 97Glu + Tyr203Asn
Gly 61Glu + Gly 95Asn + Thr207Asp
Ala 96Pro + Thr207Asp + Ser210Asp
Pro204Asn + Thr207Glu + Ser210Asp
Gly100Pro + Thr207Asp + Ser210Asp
Gly 61Asp + Thr207Asp + Ser210Asp
Asp 58Glu + Thr 64Asp + Ser 97Glu
Asn153Asp + Gly157Glu + Ala181Glu
Ser130Asp + Ser160Asp + Gly205Gln
Tyr102Asp + Ser128Asp + Ser160Asp
Gln 57Asp + Ser206Asp + Thr207Glu
Ser 99Asp + Ser126Glu + Gly152Asp
Ala181His + Asn198Glu + Thr214Glu
Gln 57Asn + Thr 64Glu + Gln200Glu
```

```
     Thr 64Asp + Leu124Thr + Gln200Asp
     Gly 98Asp + Gly125Glu + Thr207Gln
     Asn198Glu + Gln200Glu + Pro204Glu
     Asp 58Glu + Gly 59Asp + Gln200Asp
 5   Leu124Asp + Val197Asp + Asn198Asp
     Pro129Glu + Asn212Asp + Gly213Glu
     Ser158Asp + Ser210Asp + Leu211Glu
     Ser101Glu + Tyr102Glu + Gln200Asp
     Ser103Asp + Ser104Asp + Asn198Asp
10   Asn 60Glu + Ser103Glu + Ser104Glu
     Ser182Asp + Gln200Asp + Ser210Asp
     Gly157Glu + Gln200Glu + Ser210Asp
     Gln 57Asp + Gln200Asp + Ser210Glu
     Ser 99Glu + Gln200Asp + Ser210Asp
15   Ser182Glu + Asn198Asp + Gln200Glu
     Gln 57Ser + Gln200Asp + Gly213Glu
     Ser 99Glu + Ser103Asp + Ile159Met
     Ser 99Asp + Ser103Asp + Gly213Gln
     Gly155Glu + Gln185Glu + Asn198Glu
20   Gly155Asp + Gln185Glu + Ser210Asp
     Gln 57Asp + Thr 64Glu + Ser210Asp
     Thr207Glu + Leu211Glu + Gly213Asp
     Ser126Asp + Ser160Asp + Ser210Asp
     Ser130Glu + Asn198Asp + Ser210Asp
25   Ser126Asp + Asn198Asp + Ser210Glu
     Ser101Asp + Asn198Glu + Leu211Glu
     Gly155Glu + Asn198Asp + Leu211Glu
     Asp 58Glu + Asn 60Glu + Ser103Asp
     Ser 99Glu + Ser104Glu + Ser126Glu
30   Gly 59Asp + Gly 61Gln + Ser 99Asp
     Asn 60Glu + Ser158Glu + Gln185Glu
     Gly100Glu + Ser104Asp + Ser210Glu
     Gly100Asp + Ser104Asp + Ser182Glu
     Ser128Glu + Ser130Asp + Asn198Glu
35   Ser154Glu + Gln200Asp + Leu211Glu
     Ser103Asp + Gln200Glu + Leu211Asp
     Ser103Glu + Gln200Glu + Leu211Asp
     Ser126Asp + Ala156Glu + Ser182Asp
     Ser158Asp + Ser160Glu + Val197Glu
40   Ser158Asp + Ser160Asp + Val197Glu
     Val 93Gln + Gly125Glu + Ser158Glu
     Gln 57Asp + Leu124Asp + Ser126Asp
     Asn 60Asp + Ser210Glu + Asn212Glu
     Gly157Asp + Ser210Glu + Asn212Asp
45   Ser 99Glu + Ser101Glu + Ser210Glu
     Asp 58Glu + Asn198Glu + Pro204Glu
     Tyr102Asp + Ser130Glu + Ser182Asp
     Asn 60Asp + Gly 95Glu + Ser128Asp
     Leu 94Glu + Ser 97Asp + Ser206Glu
50   Gln 57Asp + Ser126Glu + Gly152Asp
     Leu 94Glu + Ser101Glu + Leu211Asp
     Gly 59Glu + Ser 97Glu + Ser182Glu
     Gly100Asp + Ser103Glu + Ser206Asp
     Asn 60Glu + Gln185Glu + Thr214Glu
```

```
       Asn  60Glu + Ser130Glu + Pro204Asp
       Asn  60Asp + Asn198Asp + Pro204Asp
       Ser  99Asp + Ser182Glu + Gln185Asp
       Pro204Asp + Ser210Glu + Leu211Gly
  5    Tyr161Val + Pro204Glu + Ser210Glu
       Ser101Glu + Asn198Asp + Gly213Glu
       Leu124Asp + Ser154Glu + Val193Ser
       Ser  99Glu + Ala181Glu + Asn212Asp
       Ala  96Glu + Gly100Asp + Ser154Glu
 10    Gly  61Asp + Ser  99Asp + Ser210Glu
       Asp  58Glu + Thr207Glu + Ser210Asp
```

TABLE 35

Multi-loop Quadruple Mutation Variants

```
 15    Gln  57Ser + Asn  60Ser + Leu  94Gly + Gln200Glu
       Val  93Gln + Tyr102Cys + Thr207Gly + Gly213Asp
       Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln
       Leu  94Gly + Ser103Glu + Gln200Asn + Ala209Gly
       Ala156His + Gln200Ser + Thr207Pro + Leu211Thr
 20    Pro129Asn + Gly155Asp + Ala209Gln + Asn212Gln
       Leu124Pro + Ala194Asn + Asn198Glu + Gln200Asn
       Gly  61Ser + Asn198Gln + Gln200Ser + Leu211His
       Tyr203Thr + Thr207Asn + Tyr208Gly + Asn212Glu
       Gln  57Asn + Asn153Asp + Leu211Ser + Gly213Pro
 25    Ala131Asn + Gln185Ser + Val193Ser + Gln200Asn
       Ala181Gly + Ala194Pro + Asn198Ser + Asn212Asp
       Ser101Asp + Leu124Val + Pro195Gln + Thr207Pro
       Gly  95Asp + Asn198Gln + Gln200Ser + Thr207Ser
       Ala  96Gln + Tyr102Pro + Ser182Glu + Val197Asn
 30    Asn  60Gln + Gly100Ser + Ser101Asp + Ile159Pro
       Gly  63Asn + Asn153Asp + Gly205Ser + Thr207Ser
       Ser126Glu + Ala131Pro + Asn153Ser + Phe183Thr
       Asn153Gln + Gly205Pro + Ser210Glu + Gly213Pro
       Ile159Glu + Ala194Ser + Pro204Gly + Leu211Ile
 35    Asp  58Glu + Val  93Ser + Ala194Pro + Gln200Ser
       Gly  95Asp + Ile105Gln + Phe183Cys + Asn198Gln
       Gly  59Pro + Gly  98Asn + Leu211Val + Thr214Asp
       Gly  61Pro + Val  93Pro + Pro129Asn + Gly213Pro
       Gln  57Asn + Ala156Pro + Ala181Pro + Phe183Gly
 40    Gly  95Gln + Gly152Asp + Phe183Met + Asn198Gln
       Gly  59Ser + Gly  95Asn + Pro127Gln + Ser210Glu
       Val197Cys + Asn198Gln + Thr207Glu + Leu211Cys
       Pro129Asn + Ser154Asp + Phe183Pro + Leu211Cys
       Gly  59Gln + Gly100Glu + Ala131Gly + Ala181Gln
 45    Gly  61Glu + Tyr161Leu + Pro204Ser + Gly213Pro
       Asp  58Glu + Ile159Met + Tyr161Gln + Gly213Asn
       Ile105Pro + Leu124Asn + Ile159Ser + Asn198Asp
       Tyr102Pro + Ala156Ser + Phe183His + Pro204Asp
       Tyr102Pro + Ile105Pro + Gln200Asn + Pro204Glu
 50    Thr  64Ser + Ile105Glu + Gln200Ser + Thr214Pro
       Gly  63Gln + Gly100Asp + Asn198Ser + Leu211His
       Asn  60Asp + Ile105Leu + Gly152Gln + Ala209Gln
```

```
        Gly152Gln + Gln185Asn + Gly205Ser + Leu211Ala
        Gln 57Ser + Gly100Pro + Pro127Gly + Asn198Glu
        Leu 94Cys + Pro127Gly + Val197Gly + Asn198Ser
        Leu 94Thr + Tyr102Cys + Leu124Cys + Tyr208Ser
 5      Ala 96Ser + Ala181Asn + Pro204Ser + Tyr208Ile
        Leu124Val + Asn198Gln + Pro204Gly + Leu211Ile
        Gly 61Asn + Ala 96Thr + Gly 98Ser + Ser210Asp
        Thr 64Pro + Ala 96Pro + Gly155Glu + Leu211Cys
        Gly157Asp + Val197Pro + Asn198Gln + Pro204Gly
10      Pro129Gln + Gly155Pro + Val197His + Ser210Glu
        Val 93Met + Ser182Asp + Gln200Ser + Gly213Ser
        Leu 94Glu + Pro127Ser + Gln200Asn + Leu211Met
        Ala 96His + Gly 98Pro + Pro129Ser + Pro204Asp
        Ser 97Asp + Gly100Pro + Leu124Pro + Thr207Asn
15      Gly 59Asn + Thr207Pro + Ser210Glu + Leu211Thr
        Ala 96Gly + Ala131Asn + Gly155Pro + Ser210Glu
        Asn153Ser + Val197Thr + Gln200Asn + Ser210Glu
        Asn 60Ser + Ile105Thr + Ser182Glu + Thr207Ser
        Gly 98Asn + Tyr161Asn + Gln200Asn + Tyr208Asn
20      Leu 94Gly + Ser 99Glu + Leu124Ser + Ala181Pro
        Ser104Glu + Ala131Pro + Leu211Ser + Gly213Ser
        Tyr102Asp + Asn198Ser + Thr207Gln + Leu211Asn
        Gly 98Pro + Ser206Glu + Leu211Gly + Gly213Pro
        Ala131Asn + Ala181Thr + Tyr208Gln + Ser210Asp
25      Gly155Gln + Tyr161Cys + Gln185Ser + Ala209Gln
        Thr 64Asn + Val 93Asn + Ser126Glu + Gln200Asn
        Ala131Pro + Phe183Ala + Thr207Asp + Thr214Gln
        Ser 97Glu + Leu124Pro + Ala131Ser + Pro204Gly
        Gly 59Asp + Tyr161His + Thr207Gly + Leu211Met
30      Gly 95Pro + Gly 98Pro + Ser101Glu + Leu211Cys
        Gly 59Ser + Thr 64Gly + Leu124Ile + Gly125Glu
        Thr 64Pro + Ser101Glu + Tyr161Gly + Gln200Asn
        Gly100Glu + Pro195Ser + Val197Ser + Thr207Asn
        Ser160Glu + Tyr161Gln + Pro204Ser + Leu211Gln
35      Leu124Met + Tyr161Leu + Ser182Glu + Leu211His
        Ile105Thr + Pro127Asn + Gly157Ser + Tyr161Pro
        Gln185Asn + Thr207Gln + Asn212Ser + Thr214Gln
        Val 93Gln + Leu 94Ser + Gly 98Glu + Thr214Gly
        Asn153Gln + Ser154Glu + Ala181Asn + Leu211Val
40      Gly125Asn + Ser154Asp + Val197Ser + Leu211Val
        Ile159Asn + Gln185Glu + Val197Met + Asn212Gln
        Ala 96Pro + Gly196Gln + Val197Ala + Asn198Glu
        Ala131Glu + Gly152Gln + Ala194Ser + Leu211Ile
        Phe183Ser + Asn198Asp + Gln200Ser + Thr207Pro
45      Gly 95Gln + Gly100Ser + Ser130Gly + Thr207Gln
        Leu124Cys + Gly152Ser + Tyr161Met + Thr207Gln
        Leu 94Met + Leu124Met + Leu211Ala + Asn212Ser
        Gly 61Asn + Ala 96Ser + Gly125Pro + Gln200Asp
        Ile159Gln + Val197Glu + Gln200Asn + Tyr208His
50      Tyr161Asn + Ser182Glu + Ala209Ser + Asn212Ser
        Gln 57Asn + Gly152Ser + Phe183Glu + Gln185Asn
        Asn198Gln + Thr207Glu + Ala209Gly + Gly213Pro
        Val197Glu + Tyr208Gly + Ala209Pro + Leu211Pro
        Ala131His + Gly205Gln + Tyr208Asp + Leu211Gln
```

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```
Gly 61Asn + Val 93Cys + Gly 98Gln + Leu211Glu
Leu124Ala + Val197Gln + Ser210Glu + Asn212Ser
Pro127Gln + Ile159Leu + Ser160Glu + Ala209Ser
Asp 58Glu + Gly 59Gln + Gly205Gln + Leu211Cys
Asp 58Glu + Phe183Tyr + Asn198Ser + Thr207Gln
Pro204Gly + Ser206Asp + Thr207Gln + Leu211His
Gln 57Asp + Gly 98Ser + Ile105Gln + Tyr208Met
Ser103Glu + Gly155Ser + Ala209His + Leu211Thr
Ser104Glu + Ala181Asn + Asn198Gln + Leu211Thr
Gln185Asn + Val197Cys + Gly205Asp + Leu211Cys
Gly 59Gln + Tyr102Glu + Pro127Gly + Ala209Thr
Ala131Thr + Asn153Ser + Val193Gln + Val197Asp
Ala131Ser + Asn153Ser + Phe183Tyr + Ala194Ser
Pro129Gln + Ala181Pro + Ala209His + Leu211Ala
Pro127Gly + Gly196Asn + Ser210Glu + Leu211Met
Leu 94Ile + Gly100Asp + Asn198Ser + Tyr208Leu
Asn153Ser + Gly155Pro + Gly205Asp + Leu211Ser
Gly 63Asn + Gly152Pro + Asn198Gln + Thr207Glu
Gly152Ser + Ser160Glu + Tyr161His + Leu211Met
Pro129Asn + Asn153Gln + Gly155Gln + Ser210Asp
Gln 57Ser + Thr 64Gln + Val197Asn + Gln200Asp
Ser126Asp + Asn153Gln + Pro204Gln + Leu211Gln
Gly 61Gln + Ala 96Gln + Phe183Leu + Leu211Thr
Gly 63Asn + Ala156Asn + Asn198Ser + Ser210Glu
Gly 59Asp + Gly152Pro + Thr207Gln + Leu211Asn
Gly 98Ser + Leu124Pro + Ile159Thr + Val197Glu
Gly205Pro + Ala209Gln + Ser210Glu + Leu211Pro
Pro129Ser + Thr207Gly + Ser210Glu + Leu211Asn
Gln 57Asp + Ala131Gln + Leu211Met + Asn212Gln
Thr 64Asn + Tyr161Ile + Phe183Asn + Ser210Asp
Gly155Gln + Pro204Ser + Ala209His + Leu211Asp
Gly100Gln + Gly157Pro + Ile159Leu + Ser160Asp
Gly 98Pro + Ser103Asp + Ala131Gly + Leu211Asn
Gln 57Ser + Gly 61Pro + Ile159Gln + Leu211Asn
Gly155Ser + Gln185Asp + Val199Met + Thr207Gly
Asp 58Glu + Ala 96Gly + Phe183Thr + Thr207Gly
Val 93Ala + Ser126Glu + Ala131Thr + Thr207Pro
Thr 64Gln + Gly 98Pro + Val197Thr + Gln200Glu
Gly152Glu + Asn153Gln + Gly155Pro + Gly213Pro
Ser154Glu + Asn198Ser + Gln200Ser + Leu211Ser
Ile105Thr + Ile159Thr + Phe183Val + Leu211Asp
Leu124Pro + Ser128Glu + Ile159Met + Leu211Pro
Gln 57Asp + Asp 58Glu + Leu211Thr + Thr214Ser
Gly 59Asp + Asn 60Asp + Val197Pro + Val199Ala
Ala 96Thr + Leu124Cys + Val197Asp + Asn198Glu
Thr 64Asn + Ala156Gly + Ser210Asp + Leu211Asp
Leu124Ser + Gln200Asn + Gly205Asp + Ser206Glu
Gly 63Pro + Ala156Asp + Gly157Asp + Ile159Gln
Thr 64Gly + Gly100Glu + Ser101Glu + Leu211Asn
Gly 61Asn + Asn153Gln + Gln200Asp + Ser210Asp
Leu124Ser + Gln200Gln + Ser210Glu + Leu211Gln
Val197Gln + Gln200Glu + Thr207Ser + Ser210Glu
Ala209Glu + Ser210Glu + Leu211Glu + Gly213Ser
Thr207Pro + Ser210Glu + Leu211Asp + Asn212Glu
```

Line numbers in left margin: 5, 10, 15, 20, 25, 30, 35, 40, 45, 50

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```
       Ser101Asp + Ser104Asp + Asn198Gln + Leu211Ser
       Thr 64Asn + Ala 96Asp + Gly 98Asp + Gly213Asn
       Asn198Glu + Gln200Asn + Ser210Asp + Leu211Thr
       Tyr161Val + Asn198Glu + Ser210Glu + Gly213Asn
  5    Val 93Pro + Asn198Glu + Gln200Ser + Ser210Glu
       Val193Pro + Asn198Asp + Thr207Ser + Ser210Asp
       Gln185Ser + Asn198Asp + Gln200Asp + Ser210Glu
       Gly 95Ser + Gly 98Gln + Asn198Asp + Leu211Asp
       Val 93His + Ala 96His + Gln200Glu + Ala209Asp
 10    Leu 94Cys + Ile159Leu + Gln200Asp + Ala209Glu
       Gln 57Asn + Gly 61Asp + Thr 64Asp + Leu 94Val
       Ala156Gln + Asn198Glu + Gly205Gln + Asn212Asp
       Tyr102Asp + Ser104Asp + Phe183Gln + Val197His
       Ala 96Ser + Asn153Ser + Gly155Asp + Ser182Asp
 15    Ala 96Asn + Asn153Glu + Gln185Glu + Thr214Gly
       Asp 58Glu + Gly 61Glu + Asn153Ser + Leu211Cys
       Pro129Ser + Asn198Asp + Gln200Asp + Leu211Pro
       Gly125Ser + Asn198Asp + Gln200Glu + Leu211Ala
       Asn198Asp + Gln200Asp + Thr207Pro + Leu211Pro
 20    Gly 98Pro + Asn198Asp + Gln200Glu + Tyr203Ser
       Asn198Glu + Gln200Glu + Tyr203Leu + Thr214Asn
       Gly100Asp + Tyr102Asp + Asn153Ser + Tyr208Leu
       Thr 64Gln + Thr207Asn + Ser210Glu + Asn212Glu
       Pro127Gln + Ile159Cys + Ser210Glu + Asn212Asp
 25    Val 93Ala + Ser 99Asp + Ser101Glu + Ile105Asn
       Gly100Gln + Gly155Gln + Asn212Asp + Thr214Asp
       Gly 95Ser + Tyr102Leu + Pro129Glu + Ser160Asp
       Gly 59Ser + Pro129Asp + Ser160Asp + Leu211Pro
       Thr 64Pro + Ala 96Thr + Asn153Glu + Ser182Asp
 30    Asp 58Glu + Gly 61Gln + Gly 95Asp + Ala181Thr
       Gly 63Pro + Ser 99Asp + Ser101Glu + Ser103Glu
       Asn153Gln + Val197Asp + Leu211Glu + Thr214Glu
       Gly152Ser + Ala181Pro + Asn198Asp + Gly213Asp
       Ser154Glu + Gly155Pro + Ser160Asp + Asn198Gln
 35    Gly152Asn + Ser154Glu + Ser158Glu + Leu211Asn
       Asn 60Glu + Leu 94Asp + Tyr208Ala + Gly213Pro
       Thr 64Asp + Gln200Asp + Ser210Asp + Gly213Pro
       Ser103Asp + Ser130Glu + Ile159Ala + Thr202Gln
       Ser103Glu + Ser130Glu + Asn198Gln + Pro204Asn
 40    Thr207Pro + Ser210Asp + Leu211Glu + Thr214Glu
       Ser210Glu + Leu211Met + Gly213Glu + Thr214Glu
       Gly125Ser + Gln200Asp + Ser206Glu + Thr207Glu
       Ala 96Pro + Gly125Asp + Ser154Asp + Ala156Asp
       Ser 99Asp + Ser104Asp + Ile105Met + Gln200Ser
 45    Gly 59Gln + Ser128Asp + Ser154Glu + Ser160Asp
       Asp 58Glu + Gly 98Asp + Leu124Ser + Tyr161Met
       Gln185Glu + Asn198Ser + Leu211Cys + Gly213Asp
       Gly 61Gln + Gly 98Pro + Gln200Asp + Thr207Glu
       Gly100Gln + Gln200Glu + Tyr203Pro + Thr207Glu
 50    Val197Pro + Gln200Asp + Thr207Asp + Ala209Gln
       Asp 58Glu + Val 93Gly + Ser 97Glu + Tyr161Ile
       Asp 58Glu + Ser 97Asp + Gln200Ser + Tyr208Thr
       Gly152Asp + Gly157Glu + Val199Ser + Thr207Ser
       Val197Gln + Pro204Ser + Thr207Asp + Ser210Asp
```

```
       Gly125Asn + Pro129Asn + Thr207Glu + Ser210Glu
       Ser101Asp + Ser128Asp + Pro129Asn + Gly152Ser
       Leu 94Ala + Asn198Glu + Gln200Asp + Thr214Glu
       Ala 96Ser + Ile105Glu + Ser126Glu + Ser160Asp
5      Ser130Asp + Ser158Glu + Ser160Asp + Asn198Gln
       Leu124Asp + Phe193Glu + Tyr208Val + Gly213Asp
       Ser101Asp + Ser126Glu + Ala156Gly + Thr207Asn
       Asp 58Glu + Asn 60Asp + Gly100Asp + Ala181Gln
       Asp 58Glu + Gly205Ser + Thr207Asp + Tyr208Val
10     Thr 64Gln + Leu 94Asp + Ser 97Asp + Gly125Asp
       Ser158Glu + Ile159Asp + Leu211Val + Gly213Glu
       Gln 57Asp + Thr 64Asp + Leu124Thr + Gln200Asp
       Gly 61Glu + Gln200Ser + Gly205Glu + Ser210Glu
       Asp 58Gln + Ile105Gly + Leu211Asp + Asn212Glu
15     Gln 57Asp + Asp 58Glu + Gln200Glu + Pro204Ser
       Gln 57Asp + Asp 58Glu + Ala 96His + Ser160Glu
       Gly157Asp + Gln200Ser + Ala209Glu + Ser210Glu
       Gly 59Glu + Asn 60Asp + Gly 95Asn + Gln185Glu
       Ser101Asp + Tyr102His + Val197Glu + Asn198Glu
20     Gly152Asp + Asn153Glu + Ser210Asp + Leu211Met
       Gly 59Asp + Tyr102Ile + Ser210Asp + Leu211Asp
       Ser101Glu + Tyr102Asp + Gln200Glu + Leu211Asn
       Gly 59Asn + Ser130Glu + Gly205Asp + Ser206Asp
       Ser128Asp + Tyr161Gly + Ser206Asp + Thr207Glu
25     Asn 60Gln + Ser 97Glu + Ser182Asp + Phe183Asp
       Gly 98Glu + Ser 99Asp + Ser160Glu + Thr207Gln
       Gly 98Asp + Thr202Gln + Thr207Glu + Tyr208Asp
       Ser154Glu + Tyr161Leu + Ser210Glu + Asn212Glu
       Asn 60Asp + Ile105Ser + Gln200Asp + Ser210Glu
30     Ala131Asp + Gln200Glu + Ser210Asp + Leu211Pro
       Gln 57Asp + Gln200Glu + Ser210Asp + Gly213Pro
       Pro129Gln + Ser160Glu + Gln200Asp + Ser210Glu
       Pro129Asp + Asn198Gln + Gln200Asp + Ser210Glu
       Gly 59Glu + Thr 64Gly + Gln200Glu + Ser210Glu
35     Asn 60Glu + Ala 96Glu + Asn198Gln + Ser210Glu
       Asn 60Asp + Ala 96Asp + Ser130Glu + Ala156Pro
       Gln 57Ser + Ile159Ser + Gln200Asp + Gly213Glu
       Gly155Asp + Gln185Glu + Asn198Glu + Tyr203His
       Ser104Glu + Leu124Asp + Gln185Ser + Val197Gly
40     Gly 59Gln + Ser101Glu + Ser103Asp + Asn198Asp
       Asn 60Gln + Ser126Asp + Ser128Asp + Ser210Glu
       Ser126Glu + Ser128Asp + Gly155Asn + Asn212Glu
       Ser103Asp + Asn198Glu + Ser210Glu + Thr214Gln
       Ser160Glu + Asn198Glu + Ser210Asp + Leu211Ser
45     Asp 58Glu + Asn198Asp + Thr207Pro + Ser210Asp
       Gly 98Asn + Ser103Glu + Asn198Glu + Ser210Glu
       Ser128Asp + Gly155Pro + Asn198Asp + Ser210Asp
       Gly152Glu + Asn198Asp + Thr207Asn + Ser210Asp
       Ser101Asp + Pro129Gly + Asn198Glu + Leu211Glu
50     Asn198Glu + Tyr208Asp + Gly213Ser + Thr214Ser
       Ser 97Glu + Gly152Glu + Ser154Asp + Gln185Ser
       Thr 64Asp + Ser210Asp + Leu211Val + Gly213Gln
       Thr 64Glu + Gly205Ser + Ser210Asp + Leu211Asn
       Leu 94Thr + Ser126Glu + Pro204Glu + Thr207Asp
```

```
      Gly100Gln + Tyr102Asp + Pro204Asp + Thr207Asp
      Ser101Glu + Asn198Glu + Gln200Glu + Leu211Ala
      Thr 64Asp + Ser 99Glu + Pro204Glu + Thr207Asn
      Gly155Pro + Ser182Asp + Gln200Asp + Leu211Asp
 5    Gly100Asn + Ser160Glu + Gln200Glu + Leu211Glu
      Asp 58Glu + Val 93Asn + Ser206Glu + Leu211Met
      Ala131Pro + Asn153Glu + Ser182Asp + Asn198Glu
      Asp 58Glu + Ala 96Glu + Pro127Ser + Gly157Glu
      Leu 94Asp + Tyr102Leu + Thr207Glu + Ala209Glu
10    Ala 96Gln + Ser158Asp + Ser160Glu + Val197Glu
      Gly100Asp + Ser126Glu + Ser154Asp + Ile159Gly
      Tyr102Asn + Gln200Glu + Thr207Glu + Gly213Asp
      Val 93Glu + Pro127Ser + Ser210Asp + Asn212Asp
      Ile105Asp + Ala131Glu + Ala156Gln + Thr202Pro
15    Gln 57Ser + Ser126Glu + Gly152Asp + Ser210Glu
      Ser126Glu + Gly152Glu + Asn198Glu + Thr207Asn
      Leu 94Glu + Ser101Asp + Tyr102Leu + Thr207Glu
      Leu 94Asp + Ser101Asp + Ser154Asp + Gly213Asn
      Asn 60Asp + Thr 64Glu + Ser103Glu + Thr214Asn
20    Asp 58Glu + Val197Asp + Ser210Glu + Leu211Pro
      Ser160Asp + Phe183Tyr + Val197Asp + Ser210Asp
      Pro129Glu + Val197Glu + Ser210Glu + Leu211His
      Ser126Asp + Ser130Glu + Ser182Glu + Gln200Ser
      Ser126Asp + Ser130Glu + Asn198Asp + Leu211His
25    Thr 64Ser + Pro204Asp + Ser210Asp + Leu211Ile
      Ser 97Asp + Gly100Glu + Gly152Pro + Asn198Glu
      Ser 97Asp + Gly100Glu + Gln200Glu + Tyr208Gly
      Asp 58Glu + Gly 59Pro + Ser154Asp + Gly157Asp
      Gly155Gln + Ala181Glu + Gln185Asp + Ser210Asp
30    Gly125Glu + Asn153Asp + Gln200Asp + Tyr208Pro
      Gln 57Ser + Ser101Asp + Ser154Asp + Ser160Asp
      Ser104Asp + Asn198Glu + Ala209Asp + Leu211Met
      Leu124Ile + Gly125Pro + Pro129Glu + Ser158Glu
      Ser154Glu + Ser158Asp + Gly205Pro + Thr207Asp
35    Gly152Glu + Ser158Asp + Gln200Asp + Thr207Pro
      Ala131Asp + Gly152Asp + Ser158Asp + Val197Thr
      Ser103Glu + Gln200Glu + Thr207Pro + Asn212Asp
      Ser130Glu + Gln200Glu + Thr202Ser + Asn212Glu
      Asn 60Glu + Leu 94Glu + Ile105Leu + Ser130Glu
40    Ser 99Glu + Pro127Asp + Pro129Ser + Ser154Glu
      Asn153Glu + Ile159Gly + Leu211Glu + Gly213Asn
      Ala156Asp + Ser160Glu + Gly205Gln + Leu211Asp
      Ser104Asp + Ser130Asp + Gly155Pro + Gly157Asn
      Gly 98Asp + Ser101Asp + Ser126Asp + Ala181Gln
45    Asn 60Asp + Tyr102Gly + Gln200Glu + Thr207Asp
      Gly 59Pro + Ala131Asp + Ser154Asp + Thr214Asp
      Ser103Glu + Ser130Glu + Asn198Glu + Asn212Gln
      Asn 60Asp + Gly 98Asn + Ser103Glu + Ser130Asp
      Ser160Asp + Phe183Ser + Ser210Glu + Thr214Asp
50    Asp 58Glu + Ser 99Asp + Val197Pro + Pro204Asn
      Asp 58Glu + Gly 98Glu + Gly155Gln + Leu211Asp
      Thr 64Glu + Leu 94Asp + Gln185Ser + Ser210Asp
      Asn 60Asp + Ser 99Glu + Ala181Glu + Val197Asn
      Ser128Gln + Ser206Asp + Ala209Glu + Asn212Gln
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Gly100Asp + Gln185Glu + Leu211Ser + Gly213Glu
Ser126Glu + Ser154Glu + Asn198Gln + Gly205Glu
Gly125Glu + Val197Thr + Gln200Glu + Thr207Asp
Asn 60Glu + Ile105Pro + Ile159Val + Leu211Glu
Pro129Asn + Gln200Glu + Pro204Asp + Thr207Gln
Asn198Ser + Gln200Asp + Tyr203Ile + Pro204Glu
Gly 98Ser + Ser103Glu + Ala131Glu + Ser210Asp
Ser103Glu + Ala131Asp + Tyr161His + Ser210Glu
Gly 61Glu + Gly125Pro + Ser126Glu + Thr207Glu
Gly152Asp + Gly157Asp + Pro204Asp + Ala209Thr
Pro127Asp + Asn153Glu + Pro204Ser + Ser206Asp
Gln 57Asp + Gln200Ser + Thr207Glu + Ser210Glu
Ser154Asp + Thr207Glu + Ser210Glu + Thr214Gln
Ser130Glu + Pro204Asn + Thr207Glu + Ser210Asp
Asp 58Glu + Val 93Ala + Ser101Glu + Ser130Asp
Gly125Glu + Ser130Asp + Gln200Asp + Leu211Thr
Leu 94Glu + Ser182Asp + Thr207Gln + Asn212Glu
Ser 97Glu + Ile159Asp + Phe183Ile + Thr214Asp
Gly 95Glu + Ser103Asp + Pro129Asn + Ala131Pro
Asn 60Gln + Gly 61Glu + Ser130Asp + Ser210Asp
Ser101Glu + Ser126Asp + Ala209Glu + Asn212Ser
Ser101Glu + Ser126Asp + Tyr161Leu + Gly205Glu
Asn 60Ser + Gly 98Asp + Ser126Glu + Leu211Thr
Val 93Glu + Ser 97Glu + Pro129Ser + Gln200Glu
Thr 64Asn + Gln200Asn + Thr207Asp + Leu211Asp
Gly 98Glu + Ser104Asp + Ser126Asp + Phe183Gly
Gly125Asp + Gly157Asp + Thr207Glu + Gly213Glu
Leu 94Asp + Ser128Asp + Gly152Asp + Leu211Met
Ser158Glu + Ser182Glu + Thr207Gly + Tyr208Cys
Gln200Asn + Pro204Gln + Ser210Glu + Thr214Glu
Thr 64Glu + Ala131Asp + Ser210Asp + Leu211Ala
Asp 58Glu + Gly 59Pro + Gly100Pro + Thr207Asp
Asn198Gln + Gln200Glu + Ser206Glu + Tyr208His
Gly 95Pro + Ser126Asp + Ala131Glu + Gln200Glu
Ser 97Glu + Ser101Glu + Gln200Asp + Pro204Asn
Ser 97Asp + Ser101Asp + Gly125Gln + Ser210Asp
Ala131Gly + Ser154Asp + Tyr161Glu + Ser210Asp
Ser126Glu + Pro204Asp + Thr207Gly + Ser210Asp
Gly 98Glu + Ser104Asp + Val197Glu + Asn198Ser
Ser104Asp + Ser130Glu + Ala156Thr + Thr207Asp
Ser104Asp + Ser130Asp + Ala131Thr + Thr207Asp
Pro127Ser + Ser158Asp + Gln200Ser + Thr214Glu
Asn 60Asp + Tyr102Ser + Val197Asn + Thr207Asp
Ser101Asp + Pro127Ser + Ala131Glu + Ala156Glu
Asp 58Glu + Ser 99Asp + Gln200Glu + Tyr208Ser
Asp 58Glu + Ser 99Asp + Leu124His + Asn198Glu
Gly 61Glu + Thr 64Ser + Val 93Asp + Tyr208Gly
Asn 60Glu + Gln185Ser + Asn198Ser + Ser206Asp
Gly 61Pro + Ser158Asp + Ala181Glu + Leu211Asp
Tyr161Glu + Gln200Glu + Tyr203His + Pro204Glu
Gly 61Glu + Gly157Asp + Leu211Met + Asn212Asp
Gly 59Ser + Gly 98Glu + Tyr102Glu + Ser158Asp
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TABLE 36

Multi-loop Quintuple Mutation Variants

Val 93Gln	+ Tyr102Cys	+ Ser154Glu	+ Asn198Gln	+ Thr207Gly
Leu 94Gly	+ Leu124Ile	+ Asn198Gln	+ Ala209Gly	+ Ser210Glu
Leu124Asp	+ Ala156His	+ Gln200Ser	+ Thr207Pro	+ Leu211Thr
Pro129Asn	+ Gly155Asp	+ Gly157Asn	+ Thr207Ser	+ Asn212Gln
Gly 61Ser	+ Gly 98Glu	+ Pro127Asn	+ Gln200Ser	+ Thr207Pro
Ala156Gln	+ Gln185Ser	+ Gln200Asn	+ Ser210Asp	+ Leu211His
Gly 63Asn	+ Tyr102Pro	+ Ala156Gln	+ Ser182Glu	+ Val197Asn
Ala 96Pro	+ Ile105Pro	+ Thr207Ser	+ Leu211Ile	+ Thr214Pro
Val 93Asn	+ Val197Gly	+ Asn198Gln	+ Ser210Asp	+ Leu211Ser
Val 93Pro	+ Leu 94Ile	+ Ile159Asn	+ Gln200Ser	+ Thr207Ser
Gly 61Pro	+ Gly 95Pro	+ Ser154Glu	+ Ala181His	+ Gly213Pro
Gly157Ser	+ Ser182Glu	+ Pro195Gln	+ Thr207Pro	+ Leu211Ser
Ser 97Glu	+ Tyr102Met	+ Asn198Ser	+ Ala209Gln	+ Leu211Ala
Leu124His	+ Ala131His	+ Gly157Pro	+ Asn198Asp	+ Thr202Ser
Gly 95Asp	+ Ala181Pro	+ Gln200Asn	+ Leu211Ala	+ Gly213Gln
Leu 94Ile	+ Gly 95Asn	+ Ser158Glu	+ Leu211Met	+ Gly213Ser
Gly 98Asn	+ Gly125Pro	+ Pro127Gly	+ Tyr161Ile	+ Tyr208Met
Ala 96Ser	+ Ser154Glu	+ Tyr161Gly	+ Gly196Gln	+ Val197Gln
Leu 94Ser	+ Gly 95Asn	+ Pro127Asn	+ Val197Ser	+ Thr214Asn
Asp 58Glu	+ Val 93Gln	+ Leu 94Met	+ Gln200Asn	+ Thr207Gln
Gly 95Asp	+ Gly155Ser	+ Val197Thr	+ Thr207Gly	+ Leu211Pro
Gly 98Ser	+ Ala131Glu	+ Val197Gln	+ Gln200Ser	+ Pro204Gly
Gln 57Asn	+ Val 93Thr	+ Tyr102Ser	+ Leu124Pro	+ Ser126Asp
Ser101Asp	+ Tyr102Cys	+ Leu124Val	+ Phe183Gly	+ Val199Asn
Asp 58Glu	+ Thr 64Gln	+ Ile105Pro	+ Gly125Gln	+ Asn198Ser
Gly155Glu	+ Tyr161Val	+ Pro204Gly	+ Thr207Pro	+ Leu211Ile
Gly100Gln	+ Ser182Asp	+ Phe183Asn	+ Asn198Ser	+ Thr207Gln
Gly 98Glu	+ Pro129Gly	+ Gly155Pro	+ Thr207Gly	+ Thr214Gly
Leu 94Met	+ Gly100Ser	+ Gly196Gln	+ Gln200Asn	+ Asn212Asp
Ser104Glu	+ Gly152Gln	+ Asn153Gln	+ Ala194Gln	+ Asn212Ser
Pro129Gln	+ Val197Thr	+ Tyr208Ile	+ Ser210Glu	+ Thr214Ser
Val 93Gly	+ Ala 96Asn	+ Pro129Gln	+ Ser160Asp	+ Pro204Gly
Pro127Gln	+ Ala131Asn	+ Gly196Ser	+ Val197Thr	+ Ser206Glu
Pro127Ser	+ Ser128Asp	+ Ala156His	+ Ala194Gln	+ Leu211Ala
Gly 95Gln	+ Pro127Gly	+ Val197Gly	+ Gly205Asn	+ Ser210Glu
Ala131His	+ Pro204Gly	+ Thr207Asn	+ Leu211Thr	+ Gly213Asn
Leu 94Gly	+ Tyr102Gln	+ Phe183Val	+ Thr207Ser	+ Leu211Asp
Gly 61Asn	+ Ile105Met	+ Gly125Ser	+ Val193Gly	+ Asn198Ser
Gly 59Pro	+ Ala 96Gly	+ Ser101Asp	+ Tyr208His	+ Leu211His
Tyr102Val	+ Gln209Asp	+ Pro204Ser	+ Leu211Ile	+ Thr214Pro
Val 93Thr	+ Ser104Glu	+ Pro195Ser	+ Val199Pro	+ Leu211Val
Ile159Gln	+ Tyr161Ala	+ Phe183Leu	+ Ser210Glu	+ Thr214Gly
Gly 59Asn	+ Pro127Ser	+ Ile159Ala	+ Gln200Asn	+ Leu211Met
Gln 57Glu	+ Val 93His	+ Gly125Gln	+ Ala131Thr	+ Ala156Asn
Ser126Asp	+ Gly157Ser	+ Asn198Gln	+ Gln200Ser	+ Thr207Ser
Gly125Asn	+ Pro129Ser	+ Phe183Asp	+ Thr207Gly	+ Leu211Gly
Asn 60Asp	+ Leu 94Met	+ Tyr102Pro	+ Gly152Asn	+ Thr207Gln
Ile105Leu	+ Gly152Pro	+ Ser158Glu	+ Asn198Ser	+ Thr214Gly
Ala 96Asn	+ Ala131Thr	+ Gly157Asn	+ Thr207Gly	+ Ser210Glu
Val 93Gln	+ Leu124Met	+ Ala181Gln	+ Asn198Glu	+ Gln200Ser
Pro127Ser	+ Ile159Thr	+ Pro195Gly	+ Asn198Ser	+ Gln200Glu

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      Ala 96Asn + Val197His + Asn198Ser + Gln200Asn + Asn212Asp
      Asp 58Glu + Leu 94Ala + Leu124Pro + Ala156Gly + Leu211Asn
      Ile105Thr + Gly157Pro + Phe183Pro + Val197Asp + Tyr208Val
      Gly 95Glu + Gly100Gln + Tyr102Gly + Tyr161His + Tyr208Cys
5     Thr 64Gly + Val 93Asp + Gly 95Pro + Gly100Pro + Asn198Gln
      Gln 57Asn + Asn 60Gln + Ser101Glu + Gly155Ser + Pro204Ser
      Gly125Ser + Gln185Asn + Asn198Gln + Ser206Glu + Ala209His
      Gly 98Ser + Pro129Asn + Val197Glu + Gln200Asn + Tyr208Asn
      Gly 98Asp + Asn153Gln + Gln200Asn + Pro204Ser + Leu211Ser
10    Ala 96Thr + Ala181Thr + Val197Asn + Gln200Asn + Thr207Pro
      Gly 95Gln + Pro129Asn + Tyr161Ala + Gln185Asp + Thr207Asn
      Gly 95Asn + Gly152Gln + Ser160Glu + Ala194Asn + Tyr208His
      Gln 57Asp + Phe183Leu + Thr207Ser + Ala209Gln + Leu211Met
      Ile105His + Gly125Asp + Gly152Asn + Pro204Asn + Tyr208Leu
15    Gln 57Asn + Leu 94Glu + Ala 96Gln + Pro127Gly + Leu211Pro
      Asn153Ser + Tyr161Met + Ala181Gln + Asn198Ser + Ser210Asp
      Gly 95Asn + Gly 98Glu + Pro129Asn + Ala209Pro + Thr214Pro
      Gly 61Asn + Asn198Ser + Ala209Glu + Leu211Ser + Gly213Pro
      Ser 99Asp + Pro127Asn + Pro195Asn + Tyr208His + Leu211Cys
20    Asn 60Gln + Thr 64Asn + Gly100Asn + Ile105Leu + Pro127Asn
      Pro127Glu + Ala194Gln + Gln200Asn + Thr207Ser + Leu211Cys
      Thr 64Gly + Leu 94Gly + Val197Asn + Gln200Ser + Ala209Pro
      Gly 59Gln + Leu 94Ser + Ala 96Ser + Leu211Thr + Thr214Glu
      Pro129Gly + Ala194Asn + Gln200Ser + Leu211Gln + Asn212Asp
25    Ala131Gly + Asn153Glu + Thr207Asn + Leu211Thr + Gly213Gln
      Gly 59Pro + Thr 64Asn + Asn198Gln + Ala209Glu + Ser210Asp
      Gly155Asn + Ser160Asp + Tyr161Glu + Asn198Ser + Leu211Ser
      Ser126Glu + Pro127Glu + Gln200Ser + Thr207Ser + Leu211Pro
      Ala131Gly + Ala209Thr + Ser210Asp + Leu211Glu + Thr214Gly
30    Gly 61Asn + Ile159Ser + Ser206Glu + Thr207Asp + Leu211Ala
      Gly 98Glu + Ser 99Glu + Tyr161Leu + Phe183Gln + Asn198Asn
      Gln 57Ser + Val 93Asn + Gly155Asn + Gln200Glu + Ser210Asn
      Thr 64Asn + Gly 95Glu + Ala 96Asp + Gly 98Asp + Gln200Ser
      Ile105Ser + Ser210Glu + Leu211Asp + Asn212Asp + Gly213Ser
35    Ala 96Asn + Gly100Ser + Ile105Cys + Asn198Asp + Ser210Glu
      Ala 96Gln + Gly 98Asn + Asn196Asp + Pro204Asn + Ser210Asp
      Gly 98Gln + Asn198Glu + Gln200Glu + Tyr208Asn + Ser210Asp
      Ile105Pro + Leu124Asn + Ile159Ser + Asn198Asp + Leu211Asp
      Leu 94Gln + Tyr161Pro + Asn198Glu + Thr207Gly + Leu211Glu
40    Tyr161Gly + Val197Ala + Asn198Gln + Pro204Glu + Ser206Asp
      Leu124Ala + Asn198Asp + Tyr208Gly + Ser210Glu + Asn212Asp
      Gln185Asn + Asn198Asp + Gln200Glu + Thr207Gln + Leu211Asp
      Pro127Gly + Tyr161Ala + Pro204Glu + Thr207Asp + Thr214Ser
      Gln 57Asn + Leu124Ser + Val197Glu + Asn198Glu + Gln200Glu
45    Asp 58Glu + Ala 96Asp + Val197Ala + Gly205Ser + Thr207Gly
      Ser158Asp + Ser160Asp + Pro195Ser + Thr207Ser + Leu211Met
      Asp 58Glu + Asn 60Glu + Thr 64Glu + Gly157Asn + Leu211Ala
      Leu124Glu + Ser126Asp + Ile159Met + Phe183Val + Val193His
      Leu 94Ile + Pro127Glu + Pro129Asp + Ser130Asp + Tyr203His
50    Leu 94Cys + Ser 99Asp + Ser101Glu + Asn198Gln + Asn212Gln
      Gly 61Asn + Ser128Glu + Ser160Glu + Ala181Asn + Thr207Gln
      Tyr161Met + Val197Asp + Gln200Asp + Ser210Glu + Asn212Gln
      Val 93Ala + Gly125Pro + Ser154Glu + Gly157Asp + Ser158Asp
      Leu 94Glu + Ser101Asp + Phe183Ala + Gln200Asn + Leu211Met
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     Leu 94Asp + Ser103Glu + Ser104Asp + Asn198Ser + Tyr208Gly
     Gly125Asp + Asn153Glu + Ser160Glu + Tyr161Asn + Asn198Ser
     Leu 94Asn + Pro127Asn + Ser154Asp + Gln185Glu + Thr214Asp
     Gly 61Glu + Thr 64Asn + Asn198Glu + Ser210Asp + Gly213Asp
 5   Leu124Gln + Asn198Glu + Gln200Asp + Thr207Gly + Tyr208Glu
     Leu 94Glu + Leu124Asp + Pro129Asn + Ala156Ser + Thr207Gly
     Gly 98Asn + Ala156Pro + Asn198Glu + Gln200Asn + Gly213Asp
     Gly 95Glu + Gly 98Gln + Ser 99Glu + Tyr102Cys + Ser104Glu
     Gly125Asn + Gln200Asp + Thr207Glu + Ser210Asp + Leu211His
10   Gly 59Asp + Ser 97Asp + Ser 99Asp + Ala131Pro + Pro204Gly
     Gln 57Ser + Tyr102Gln + Asn198Glu + Ala209Glu + Leu211Ser
     Asn153Asp + Ile159Asp + Tyr161His + Phe183His + Gly213Pro
     Gly100Ser + Gly155Asp + Ile159Glu + Val197Met + Thr207Pro
     Pro127Ser + Gln200Asp + Thr207Gly + Ser210Asp + Gly213Glu
15   Ala 96Thr + Ser126Glu + Gly152Glu + Ser158Glu + Thr207Asn
     Leu 94Ala + Gly 95Asp + Ser 97Asp + Ser101Asp + Thr202Gly
     Gly152Ser + Gly205Ser + Thr207Glu + Ser210Glu + Leu211Asp
     Tyr161Pro + Pro195Ser + Thr207Asp + Ser210Glu + Leu211Asp
     Asp 58Glu + Ser 97Glu + Ser 99Asp + Gln185Asn + Gly196Ser
20   Thr 64Gly + Ser101Glu + Ile105Glu + Ser126Asp + Tyr161Leu
     Thr 64Gly + Ser 99Asp + Ile105Glu + Gly205Pro + Leu211Ala
     Asn153Glu + Ala194Thr + Asn198Glu + Tyr208Ile + Gly213Glu
     Gly152Gln + Gln200Asn + Ser206Glu + Ala209Asp + Ser210Glu
     Ser103Glu + Gly125Pro + Ala131Glu + Tyr161Cys + Leu211Thr
25   Gly100Ser + Gln200Asn + Thr207Glu + Ser210Asp + Leu211Glu
     Gly100Pro + Asn198Asp + Gln200Glu + Gly205Gln + Thr207Glu
     Ala 96Asp + Ser 99Glu + Ser104Asp + Val197Met + Gly213Ser
     Gly 98Asp + Gly100Pro + Ser101Asp + Gly125Ser + Ala156Ser
     Gly 98Asp + Gly125Asp + Ser160Glu + Tyr161Ile + Thr214Gly
30   Gly 61Glu + Gly155Gln + Asn198Gln + Ser206Glu + Tyr208His
     Asp 58Gln + Asn 60Glu + Leu124Asn + Ser206Asp + Thr207Asn
     Gly 61Asp + Thr202Pro + Thr207Gln + Ser210Glu + Leu211Pro
     Gly 61Glu + Tyr102His + Phe183His + Gln185Asn + Ser210Asp
     Ser 99Asp + Ser126Asp + Pro129Asp + Gly152Ser + Leu211His
35   Leu 94Ile + Gly125Gln + Ser128Asp + Pro129Glu + Ser154Asp
     Asn198Asp + Gln200Ser + Gly205Pro + Gly213Ser + Thr214Asp
     Gly 61Asn + Ser162Asp + Val193Gln + Asn198Asp + Ser210Asp
     Gly 61Glu + Leu 94Cys + Gln200Ser + Gly205Glu + Ser210Glu
     Asp 58Glu + Phe183Ser + Pro204Glu + Thr207Ser + Ser210Glu
40   Leu 94Met + Ala156Asp + Tyr161Cys + Asn212Glu + Thr214Glu
     Asp 58Glu + Leu124Pro + Ile159Thr + Leu211Asp + Asn212Glu
     Gln 57Glu + Leu 94Thr + Val197Ser + Ala209Glu + Ser210Asp
     Ser104Glu + Pro129Ser + Ala156Gly + Ala209Glu + Ser210Glu
     Asn153Asp + Ser154Glu + Asn198Asp + Thr207Gln + Leu211Gln
45   Gly 59Asp + Asn 60Glu + Ser101Glu + Tyr102His + Thr207Asn
     Gly 61Gln + Ser104Asp + Ile105Asp + Asn153Ser + Thr207Asp
     Ser 97Glu + Val197Gln + Asn198Gln + Asn212Asp + Gly213Asp
     Tyr102Val + Ser154Glu + Gly205Glu + Ser206Asp + Leu211Cys
     Asn153Asp + Tyr161Thr + Pro195Gly + Ser206Asp + Thr207Gln
50   Gly 59Gln + Thr 64Pro + Gly 98Glu + Ser130Asn + Ala131Glu
     Gly125Pro + Gly152Asn + Ser158Glu + Ile159Glu + Ser210Glu
     Ser 97Asp + Gly 98Asp + Ile105Val + Leu124Thr + Gln200Asp
     Gly100Glu + Ser101Asp + Ser160Asp + Val197Ala + Leu211Gln
     Ser103Asp + Ser104Asp + Ala181Ser + Asn198Asp + Tyr208Ile
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     Ser 99Glu + Gly100Glu + Gly125Asn + Ser182Asp + Thr207Gly
     Leu 94Gly + Gly100Asp + Ala131Gly + Gln200Glu + Ser210Asp
     Ser 97Glu + Ala131Gln + Gln200Asp + Thr207Gly + Ser210Glu
     Gly125Asn + Ser130Glu + Phe183Asn + Gln200Asp + Ser210Glu
5    Gly 95Gln + Tyr102Leu + Pro127Glu + Gln200Asp + Ser210Asp
     Gly155Asn + Gly155Asn + Gln200Glu + Ser210Asp + Thr214Gly
     Gln 57Ser + Asp 58Glu + Gln200Asp + Thr207Gly + Ser210Glu
     Pro127Asp + Tyr161Cys + Gln185Asn + Gln200Asp + Ser210Asp
     Gly100Glu + Gln200Asp + Thr207Pro + Ser210Asp + Leu211Ala
10   Gly 59Pro + Ser160Asp + Asn198Gln + Gln200Asp + Ser210Glu
     Thr 64Glu + Ile105Cys + Leu124Ser + Val197Glu + Ser210Asp
     Gln 57Glu + Val 93Glu + Gly 95Ser + Ser103Glu + Asn198Gln
     Asn 60Glu + Ala 96Asp + Tyr102Glu + Gln200Ser + Thr207Gly
     Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu + Gly213Asp
15   Asn198Asp + Pro204Gln + Ser206Glu + Ser210Glu + Leu211Thr
     Val 93Glu + Ser126Glu + Ser160Asp + Val197Gln + Leu211Ser
     Gly 98Asp + Ser126Glu + Pro129Asp + Ala181Ser + Gly213Ser
     Ala 96Glu + Gly 98Glu + Ser103Asp + Pro127Ser + Ile159Pro
     Gly 63Gln + Ser128Glu + Gly155Ser + Asn198Glu + Ser210Glu
20   Gln 57Asp + Ala 96Thr + Gln185Asn + Asn198Asp + Ser210Asp
     Asn 60Gln + Gly 95Asp + Asn198Glu + Gln200Asn + Ser210Asp
     Ser104Asp + Ile105Met + Asn198Asp + Ser210Asp + Leu211Asn
     Tyr102Glu + Asn198Asp + Gln200Asn + Ser210Asp + Leu211Val
     Gln 57Asp + Gly125Ser + Asn198Asp + Gly205Asn + Ser210Asp
25   Tyr102Leu + Gln185Glu + Asn198Glu + Gln200Ser + Ser210Glu
     Gly 59Asp + Tyr102Ile + Asn198Glu + Ser210Asp + Thr214Ser
     Val 93Thr + Leu124Val + Ile159Glu + Asn198Asp + Ser210Glu
     Ser101Asp + Pro129Gly + Asn198Glu + Thr207Ser + Leu211Glu
     Gly152Gln + Gly155Gln + Asn198Asp + Gly205Asn + Leu211Asp
30   Gly 59Gln + Thr 64Ser + Ala156Asp + Ser158Glu + Gly205Glu
     Pro127Asp + Asn198Ser + Pro204Glu + Ser206Asp + Thr207Pro
     Asp 58Glu + Ile159Gly + Asn198Asp + Leu211Ile + Asn212Glu
     Ser130Asp + Ile159Gly + Asn198Glu + Leu211Met + Asn212Glu
     Asp 58Glu + Asn 60Glu + Ile159Met + Tyr161Gln + Gln200Asp
35   Ser 97Asp + Ser103Glu + Ile105Glu + Val197Cys + Leu211Val
     Ser103Glu + Ile105Glu + Asn153Ser + Gln200Ser + Ser210Asp
     Ala 96Pro + Gly152Asp + Ser160Asp + Thr207Glu + Leu211Gln
     Gly 95Asp + Ser 99Glu + Ile105Gly + Gly157Pro + Leu211Glu
     Ser 97Asp + Ser 99Glu + Val197Glu + Asn198Ser + Thr214Gly
40   Gln 57Asn + Ser 97Glu + Ser 99Glu + Asn153Glu + Val193His
     Thr 64Glu + Ala131Asn + Tyr208Leu + Ser210Asp + Gly213Ser
     Gln 57Glu + Ala156Pro + Ser158Asp + Gln185Glu + Thr207Gln
     Ser 97Asp + Pro129Asn + Ser158Glu + Gln185Glu + Leu211Ser
     Ala 96Thr + Ala156His + Ser158Glu + Gln185Asp + Leu211Asp
45   Leu 94Ala + Ser101Asp + Ser126Glu + Ala131Gln + Ser154Asp
     Gly157Glu + Ile159Leu + Gln200Asn + Pro204Asp + Thr207Glu
     Leu 94Thr + Ser126Glu + Pro204Glu + Thr207Asp + Gly213Asn
     Gly100Glu + Ser154Glu + Asn198Glu + Gln200Asp + Leu211Ser
     Gln 57Glu + Asn198Glu + Gln200Glu + Tyr203Val + Gly213Pro
50   Ile105Met + Ser156Glu + Ile159Ser + Asn198Glu + Gln200Asp
     Ser104Asp + Ala181Gln + Asn198Glu + Gln200Asp + Thr207Gly
     Gly100Asn + Gly152Pro + Ser182Asp + Gln200Asp + Gly213Asp
     Tyr102Met + Ser128Glu + Ser130Asp + Asn198Asp + Leu211Met
     Ser128Glu + Ser130Glu + Ala131Thr + Asn198Glu + Asn212Gln
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      Val 93Thr + Leu124Glu + Ile159Asn + Gln200Glu + Leu211Asp
      Gly157Ser + Ser182Asp + Gln200Glu + Thr207Pro + Leu211Glu
      Ser130Glu + Gln200Asp + Gly205Gln + Thr207Gln + Leu211Glu
      Gln 57Asn + Gly 59Asn + Asn 60Glu + Gly 98Glu + Ser130Asp
  5   Gly 61Asp + Tyr102Pro + Ser206Glu + Thr207Asn + Ser210Glu
      Thr 64Asn + Ser 97Asp + Pro129Asn + Val197Asp + Leu211Asp
      Ser 99Glu + Ala181Thr + Val197Glu + Ala209Asn + Leu211Asp
      Gly 59Asp + Val 93Ser + Asn153Ser + Val197Glu + Leu211Asp
      Ser101Glu + Asn153Ser + Tyr208Met + Leu211Glu + Gly213Asp
 10   Gly157Asp + Gln185Asn + Thr207Glu + Ala209Asp + Asn212Gln
      Gly100Gln + Gly125Ser + Phe183Asp + Ser210Asp + Thr214Glu
      Asn198Gln + Ser206Glu + Thr207Pro + Ser210Asp + Asn212Asp
      Ala181Thr + Gln200Glu + Thr207Asp + Leu211Ala + Gly213Glu
      Asn 60Asp + Gly 98Pro + Ser126Glu + Pro129Glu + Gln200Ser
 15   Asp 58Glu + Ile159Pro + Ser210Glu + Asn212Asp + Thr214Gln
      Asp 58Glu + Gly125Ser + Ala131Ser + Ser210Asp + Asn212Glu
      Gly 59Ser + Ser101Glu + Ala131His + Ser210Glu + Asn212Glu
      Asn 60Gln + Val 93Ala + Ser103Glu + Ser210Glu + Asn212Glu
      Ser158Glu + Asn198Ser + Tyr208Glu + Ser210Asp + Leu211Ser
 20   Ala 96Ser + Ser160Asp + Asn198Glu + Asn212Ser + Thr214Glu
      Leu 94Asn + Gly 98Asp + Gly100Glu + Pro127Glu + Val197Met
      Asn 60Ser + Gly 98Asp + Gly100Asp + Tyr102Asn + Gln200Glu
      Leu 94Ser + Ala 96Thr + Ile105Asp + Tyr161Glu + Leu211Ile
      Gly 95Ser + Ser 99Asp + Ser101Asp + Leu211Met + Gly213Glu
 25   Gly 61Glu + Gly125Ser + Ser128Glu + Ser160Asp + Ala181Pro
      Ser182Glu + Val193Met + Asn198Glu + Gly205Gln + Ala209Asp
      Leu 94Glu + Gly100Asn + Ser104Asp + Ile105Val + Ser206Glu
      Thr 64Asp + Ser126Asp + Gly152Asp + Pro204Gly + Leu211Asn
      Gly 59Asp + Val 93Glu + Leu 94Pro + Ile105Met + Leu211Ser
 30   Pro129Glu + Ser160Asp + Gln200Ser + Gly205Gln + Ser210Glu
      Ile159Val + Ser160Glu + Phe183Glu + Pro204Gln + Ala209Asn
      Leu 94Asp + Val197Asp + Thr207Gly + Ser210Asp + Leu211Met
      Leu124Glu + Phe183Pro + Val197Glu + Tyr208Leu + Ser210Glu
      Leu 94Met + Leu124Asp + Asn153Asp + Tyr161Ala + Leu211Glu
 35   Gly 98Asp + Gly152Glu + Ala156Asp + Tyr208Met + Asn212Ser
      Val 93Asp + Gly 98Gln + Ser126Asp + Ser130Glu + Ala209Pro
      Asp 58Glu + Tyr102Ala + Ser126Asp + Ser130Glu + Ile159Asn
      Ser126Asp + Ser130Glu + Val197Gly + Leu211Glu + Asn212Ser
      Ser104Glu + Ile105Pro + Asn198Asp + Leu211His + Gly213Asp
 40   Ser 97Asp + Gly100Glu + Gly152Pro + Tyr161Glu + Ala181Gln
      Ser128Glu + Ala131Glu + Ser154Asp + Asn198Gln + Ala209His
      Ser 99Asp + Pro129Ser + Ser154Glu + Ser160Asp + Pro204Gln
      Asp 58Glu + Ile105Cys + Leu124Ser + Ser154Glu + Ser158Glu
      Ala131Asp + Val197Glu + Gln200Glu + Thr207Pro + Thr214Gly
 45   Ser 99Asp + Val197Asp + Asn198Gln + Gln200Glu + Thr207Gly
      Ser101Glu + Val197Asp + Gln200Glu + Pro204Gly + Thr207Ser
      Ala131Asp + Val197Asp + Asn198Gln + Gln200Glu + Ala209Asn
      Gly 95Pro + Gly 98Asp + Leu124Glu + Thr207Asn + Asn212Glu
      Gln 57Glu + Asn 60Glu + Val 93Thr + Gly 95Asn + Ser182Asp
 50   Asn 60Asp + Ile105Gln + Ala131Thr + Thr207Ser + Ala209Glu
      Ser103Glu + Gln200Glu + Thr207Pro + Ala209Gln + Asn212Asp
      Gly 61Pro + Leu 94Glu + Gln200Asp + Leu211Asn + Asn212Glu
      Gly 59Pro + Val 93Glu + Gln200Asp + Leu211Val + Asn212Asp
      Ser 97Glu + Gly157Ser + Ala181Pro + Gln200Glu + Asn212Glu
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     Val  93Thr + Gly  98Gln + Tyr102Glu + Ala131Glu + Ser210Asp
     Gly  61Glu + Ser104Asp + Phe183Ala + Thr207Gly + Ala209Glu
     Ala156Asp + Ser160Asp + Ala181Gly + Asn198Ser + Gln200Asp
     Gln  57Glu + Gly  61Asp + Asn153Gln + Asn198Asp + Gly205Ser
  5  Gly100Glu + Ile105Ser + Leu124Glu + Val197Met + Ser210Asp
     Tyr102Glu + Ser126Glu + Val193Pro + Pro204Asn + Ser210Asp
     Gly  59Pro + Ala131Asp + Asn153Ser + Ser154Asp + Thr214Asp
     Ser103Glu + Ser130Glu + Gln200Ser + Gly205Glu + Thr214Gly
     Asp  58Asp + Ala156Pro + Gly157Asp + Ser160Asp + Gln200Ser
 10  Thr  64Glu + Leu  94Asp + Ala  96Gln + Gln185Ser + Ser210Asp
     Ser130Asp + Gly157Asp + Phe183Glu + Asn198Gln + Leu211Met
     Asp  58Glu + Pro129Glu + Ala156His + Pro204Ser + Gly205Glu
     Ser126Glu + Ile159Leu + Ala209Asp + Leu211Ala + Asn212Glu
     Gly  59Asp + Ser126Asp + Ser154Glu + Phe183Leu + Leu211Val
 15  Gly155Asn + Gly157Asp + Gln200Glu + Thr207Glu + Leu211Asn
     Gly  59Glu + Gly  95Pro + Ala181His + Gln200Glu + Thr207Asp
     Gln  57Glu + Pro127Gly + Gly152Ser + Gln200Asp + Thr207Asp
     Ala  96Glu + Asn198Ser + Gln200Glu + Thr207Glu + Thr214Ser
     Thr  64Asp + Leu124Ser + Pro129Asp + Tyr161Asn + Thr207Asp
 20  Ser  97Asp + Tyr161Gly + Ser210Glu + Leu211Pro + Gly213Asp
     Pro127Ser + Pro129Asn + Gly157Asp + Ser210Glu + Gly213Glu
     Tyr102Cys + Ser130Asp + Pro204Gln + Ser210Glu + Gly213Asp
     Ser  99Glu + Ala156Ser + Ala209Thr + Ser210Asp + Gly213Asp
     Gly196Pro + Gln200Asp + Pro204Asp + Thr207Gln + Leu211Asn
 25  Asp  58Asn + Ser  97Glu + Ser104Ser + Pro129Gly + Tyr161Thr
     Asp  58Glu + Leu  94Ser + Ser  97Asp + Gly  98Pro + Gln200Asp
     Gly  61Glu + Ser101Asp + Thr207Asp + Leu211Ile + Thr214Ser
     Gly  61Asp + Ser101Glu + Tyr102Val + Thr207Glu + Thr214Ser
     Gly  61Asp + Asn153Gln + Ile159Gly + Asn198Asp + Thr207Glu
 30  Gly  61Glu + Ile159Ser + Val197Met + Asn198Glu + Thr207Asp
     Thr  64Gln + Gly100Glu + Gln200Asn + Thr207Glu + Ser210Asp
     Ser126Glu + Gly155Pro + Tyr161Thr + Thr207Glu + Ser210Asp
     Tyr102Glu + Gly125Gln + Ala181Pro + Thr207Asp + Ser210Glu
     Gly100Glu + Gly205Ser + Thr207Glu + Ser210Glu + Asn212Gln
 35  Gly  59Ser + Gly  95Glu + Thr207Glu + Ser210Asp + Leu211Val
     Tyr161Asp + Asn198Ser + Thr207Glu + Ser210Glu + Gly213Pro
     Pro129Gln + Ser130Glu + Asn198Gln + Thr207Asp + Ser210Glu
     Gln  57Asp + Phe183Gly + Pro204Gln + Thr207Glu + Ser210Asp
     Asn  60Gln + Gly157Glu + Pro204Gly + Thr207Asp + Ser210Asp
 40  Pro129Asp + Gly152Pro + Thr207Asp + Ser210Glu + Leu211Asn
     Gly  61Glu + Ser  99Asp + Gly155Asn + Pro195Gln + Gln200Asn
     Gly  98Glu + Ser101Glu + Ala181Gly + Gln185Glu + Thr207Pro
     Gly  59Pro + Thr  64Asp + Pro129Gln + Ala181Glu + Asn198Asp
     Gly  98Asp + Tyr161Met + Ala181Glu + Asn198Asp + Leu211Val
 45  Ile159Glu + Val197Thr + Tyr208Gln + Ala209Glu + Thr214Asp
     Ser126Glu + Gly157Glu + Ser182Glu + Asn198Gln + Leu211Val
     Gly  61Asp + Ser126Glu + Ser206Asp + Tyr208Gly + Leu211Met
     Gly  98Pro + Ser130Glu + Ser160Glu + Thr207Gly + Ser210Asp
     Gln  57Asp + Gly125Gln + Ser130Asp + Asn153Ser + Ser160Asp
 50  Tyr161Met + Gln200Glu + Ser206Gln + Ala209His + Gly213Asp
     Gly  61Asp + Ala156Glu + Gly205Asn + Thr207Gln + Ser210Glu
     Ile105Glu + Ser130Asp + Gln200Asn + Gly205Glu + Thr207Asn
     Gln  57Ser + Asn  60Asp + Gln200Asp + Gly205Glu + Thr207Ser
     Ser101Glu + Ser126Glu + Val197Asp + Thr207Gly + Leu211Cys
```

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      Ser101Asp + Ser126Asp + Gly152Ser + Asn198Glu + Gln200Asn
      Gln 57Asp + Gly125Asp + Gly205Asp + Thr207Ser + Tyr208Thr
      Leu 94Met + Ser128Asp + Ser158Glu + Asn198Gln + Ser210Asp
      Gly 59Asp + Gly 95Pro + Ala194Gln + Asn198Asp + Thr214Asp
 5    Gln 57Glu + Gly 61Asn + Gly 98Asp + Gly100Pro + Val193Cys
      Thr 64Gln + Val 93Glu + Ser 97Glu + Pro129Ser + Gln200Glu
      Gln 57Glu + Thr 64Asn + Val 93Asp + Gln200Glu + Thr207Asn
      Gly100Asp + Gly125Ser + Ser126Glu + Gly196Gln + Val197Glu
      Val 93Met + Gly100Asp + Ser126Glu + Tyr161Ala + Asn198Asp
10    Gly100Glu + Ser126Asp + Ala181His + Val197Cys + Gln200Glu
      Asn 60Gln + Ser 99Asp + Ser103Glu + Ser210Asp + Leu211Ala
      Gln 57Ser + Ser 99Glu + Ser103Glu + Gln200Asp + Thr207Asn
      Gly 63Ser + Ala 96Ser + Ser210Gln + Leu211Pro + Thr214Asp
      Pro129Gly + Val197Gln + Ser210Glu + Leu211His + Thr214Asp
15    Pro127Asp + Gly155Glu + Tyr161Ser + Thr207Ser + Ser210Glu
      Asp 58Glu + Val 93His + Gly155Pro + Gln200Asn + Thr207Glu
      Thr 64Asn + Asn198Ser + Gln200Glu + Ser206Glu + Thr214Gln
      Gly 59Pro + Gly 61Glu + Ser154Asp + Gln200Asp + Thr214Asn
      Ser126Asp + Pro129Ser + Ala156Asp + Ser210Asp + Leu211Ala
20    Ser103Glu + Ile159Leu + Pro204Glu + Gly205Asn + Ser210Asp
      Ala131Thr + Asn153Glu + Tyr203Asn + Pro204Glu + Ser210Glu
      Gly157Asn + Ser160Glu + Pro204Glu + Gly205Ser + Ser210Glu
      Gly 95Gln + Gly 98Pro + Gly152Glu + Gly157Pro + Val197Asp
      Gln 57Glu + Leu 94Pro + Gly125Ser + Asn153Glu + Leu211Glu
25    Gly 59Ser + Ser104Asp + Ser130Glu + Ser210Glu + Leu211Ser
      Ser104Glu + Ser130Asp + Gly155Asn + Leu211Gly + Gly213Asp
      Ser104Glu + Ser130Glu + Asn198Asp + Gln200Asn + Thr207Gln
      Ser158Glu + Thr202Ser + Thr207Pro + Leu211Thr + Thr214Asp
      Gly 59Glu + Leu 94Val + Ser101Asp + Ala131Glu + Tyr161Met
30    Asp 58Glu + Ser 99Glu + Tyr102Asn + Ala209Asn + Ser210Glu
      Ser101Asp + Phe183Ser + Gln200Asp + Pro204Glu + Thr214Gln
      Gly 61Glu + Gly 98Asn + Ser 99Glu + Ile105Ser + Val197Glu
      Asn 60Ser + Gly125Pro + Ala156Asp + Tyr161Asp + Ser210Asp
35    Gly 59Pro + Ser128Glu + Ser154Asp + Thr207Glu + Leu211Ile
      Ser103Asp + Ala156Gly + Ser182Glu + Asn198Glu + Tyr203His
      Val 93Ala + Gly125Pro + Ser160Glu + Thr207Asp + Leu211Glu
      Ser 97Asp + Gly152Pro + Thr207Glu + Leu211Glu + Gly213Gln
      Gln 57Glu + Ser103Asp + Pro129Glu + Ala181Gly + Thr207Pro
      Ser158Asp + Tyr161Pro + Ser182Asp + Thr207Gln + Ser210Asp
40
```

TABLE 37

Multi-loop Sextuple Mutation Variants

```
      Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln
         + Thr207Gly
45    Ser160Glu + Tyr203Cys + Thr207Pro + Leu211Met + Asn212Gln
         + Gly213Gln
      Val 93Ser + Ser103Glu + Gly125Ser + Gln185Asn + Ala194Gln
         + Asn198Gln
      Gly100Ser + Ile105Met + Gly157Pro + Gln200Asn + Thr207Asn
50       + Leu211Val
      Thr 64Glu + Leu 94Asn + Val197Cys + Thr202Gln + Thr207Gln
         + Leu211Val
```

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Gly152Pro + Gly157Gln + Ala181Asp + Asn198Gln + Gln200Asn
    + Thr207Asn
Gly 95Gln + Ala 96Asn + Leu124Thr + Asn153Ser + Ser154Glu
    + Val197His
5 Val 93Met + Pro127Ser + Gly155Pro + Ser182Asp + Gln200Ser
    + Gly213Ser
Gly 61Pro + Gly 95Pro + Ser154Glu + Ala181His + Leu211Asn
    + Gly213Pro
Tyr161Thr + Pro195Asn + Gln200Asn + Thr207Gly + Tyr208Leu
10   + Leu211Pro
Leu 94Pro + Ala 96Ser + Pro127Asp + Ile159Ala + Thr207Pro
    + Gly213Gln
Gly100Asn + Ile105Gln + Gly125Gln + Pro129Gly + Gln185Glu
    + Thr207Gln
15 Gly 98Glu + Pro129Gly + Gly155Pro + Thr207Gly + Ala209His
    + Thr214Gly
Ile105Gln + Gly152Asp + Phe183His + Ala194Gln + Gly205Ser
    + Asn212Gln
Ala194Gln + Asn198Ser + Gln200Asn + Ser206Asp + Leu211His
20   + Thr214Gln
Ser 99Glu + Pro129Asn + Ala131Pro + Gln185Asn + Thr207Pro
    + Leu211Val
Leu 94Val + Gly 98Gln + Leu124Gly + Val197Glu + Gln200Asn
    + Thr207Gly
25 Gln 57Ser + Gly 98Asn + Tyr102Asp + Tyr161Asn + Gln200Ser
    + Thr207Gln
Asn 60Gln + Gly 61Ser + Tyr102Cys + Gly152Asp + Val199Ser
    + Pro204Asn
Tyr102Thr + Gly155Glu + Ala156Asn + Tyr161Pro + Gln200Asn
30   + Leu211Thr
Tyr102Glu + Gly157Pro + Phe183His + Asn198Gln + Thr207Pro
    + Leu211Cys
Leu124Cys + Pro127Asn + Tyr208Met + Ser210Asp + Leu211His
    + Gly213Gln
35 Gly 98Ser + Leu124Ala + Ser126Asp + Ala181Asn + Ala209Gly
    + Gly213Gln
Ala 96His + Tyr102Ser + Ile105Ser + Gly157Ser + Ile159Gln
    + Leu211Asp
Thr 64Pro + Ala 96Pro + Ser 97Asp + Pro127Ser + Gly205Asn
40   + Leu211Ser
Val 93Gly + Gly 98Ser + Tyr102Asn + Val197Met + Thr207Gln
    + Ser210Asp
Asn 60Gln + Ala 96Gln + Asn153Glu + Thr202Gln + Gly205Asn
    + Leu211Met
45 Gly 59Asn + Leu 94Val + Pro127Ser + Ile159Ala + Gln200Asn
    + Leu211Met
Ile105Gln + Gly157Gln + Val193Asn + Val197Asp + Thr207Pro
    + Tyr208Cys
Asn 60Gln + Gly 63Gln + Ile105Glu + Asn198Gln + Leu211Ile
50   + Thr214Gly
Gly 61Gln + Gly125Glu + Tyr161Gly + Gly205Gln + Thr207Ser
    + Ala209Gln
Gly 98Ser + Ala156Gln + Tyr161Ala + Ser182Glu + Gln200Ser
    + Thr207Gln

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Gly 61Pro + Val 93Ala + Leu124Gly + Ala131Thr + Val197Glu
    + Asn198Gln
Val 93Asp + Ala 96Asn + Val197His + Asn198Ser + Val199Ser
    + Thr207Gln
5   Tyr102Ile + Ile159Asp + Ala181Asn + Gln200Ser + Pro204Gln
    + Leu211His
Gly100Pro + Pro129Ser + Gln200Ser + Thr207Pro + Ala209Glu
    + Ser210Glu
Val197Pro + Asn198Gln + Gln200Glu + Pro204Gly + Thr207Gln
10 + Ser210Asp
Ala 96Gln + Leu124Met + Ile159Ser + Gln200Glu + Pro204Gly
    + Ser210Asp
Asn 60Gln + Ile159Thr + Asn198Ser + Gln200Glu + Ser210Glu
    + Leu211Asp
15 Gly 61Ser + Val197Gln + Asn198Glu + Gln200Ser + Ser210Asp
    + Leu211His
Gly100Asn + Ala181Pro + Asn198Asp + Thr207Ser + Ser210Glu
    + Gly213Gln
Gly 98Asn + Pro129Ser + Asn198Asp + Gln200Asp + Ser210Glu
20 + Leu211Pro
Gly 63Ser + Leu 94Gln + Ile159Ala + Gly205Glu + Thr207Glu
    + Thr214Asn
Asp 58Glu + Gly 61Glu + Gly 98Asn + Asn153Ser + Tyr161Asn
    + Leu211Cys
25 Leu124Ser + Ile159Thr + Asn198Glu + Gln200Glu + Pro204Gly
    + Thr207Gln
Gly 61Gln + Gly 95Asn + Ala194Gly + Asn198Asp + Gln200Asp
    + Leu211Val
Gln 57Ser + Tyr102Gln + Asn198Glu + Ala209Glu + Ser210Glu
30 + Leu211Ser
Gly 59Gln + Gly125Pro + Ser154Glu + Gly155Asp + Tyr161Ala
    + Ser182Asp
Gln 57Glu + Gly 59Asp + Thr 64Gln + Tyr208Pro + Ala209Gln
    + Leu211Asn
35 Asn 60Asp + Gly 61Gln + Gly 95Glu + Gly 98Glu + Asn153Ser
    + Ile159Leu
Ile105Thr + Gly152Ser + Ser156Asp + Ser160Asp + Thr207Gly
    + Leu211Ala
Gly 61Gln + Thr 64Asn + Asn198Glu + Ser210Asp + Leu211Asp
40 + Gly213Asp
Leu 94Pro + Ala 96Gly + Thr207Ser + Ser210Asp + Leu211Cys
    + Asn212Glu
Gly 96Glu + Gly100Asp + Ala181Pro + Tyr203Val + Pro204Asn
    + Thr207Ser
45 Asp 58Glu + Gly157Ser + Gln185Asn + Val197Gln + Pro204Glu
    + Asn212Gln
Gly 61Asn + Leu124Thr + Ser128Glu + Ser160Glu + Ala181Asn
    + Thr207Gln
Ile105Leu + Leu124Ser + Gly125Asp + Gly152Glu + Asn153Glu
50 + Gly213Glu
Leu124Gln + Asn198Glu + Gln200Asp + Thr207Gly + Tyr208Glu
    + Leu211Asp
Leu 94Ile + Gly152Ser + Gly155Glu + Ser158Asp + Ala181Gly
    + Val197His

Asp 58Glu + Tyr161Gly + Val197Ala + Asn198Gln + Pro204Glu
    + Ser206Asp
Gln 57Ser + Ile159Ser + Asn198Asp + Gln200Asn + Tyr208Asn
    + Gly213Glu
Gly152Ser + Phe183Ala + Asn198Glu + Val199Ser + Leu211Cys
    + Gly213Glu
Leu 94Ile + Ser154Asp + Ile159Glu + Asn198Ser + Gln200Asn
    + Leu211Met
Ala 96Pro + Ser154Asp + Ala156His + Gly157Asn + Ser158Glu
    + Pro204Ser
Gln 57Asp + Gly 59Pro + Asn 60Asp + Gly 95Pro + Thr207Ser
    + Ala209Gln
Thr 64Ser + Gly152Gln + Ala181His + Gln200Glu + Leu211His
    + Asn212Glu
Gln 57Asn + Gly 98Gln + Gln200Asp + Leu211Cys + Asn212Asp
    + Gly213Glu
Ala 96Asn + Gly100Asp + Leu124Asp + Val197Gly + Thr207Pro
    + Gly213Ser
Ala 96Ser + Ser154Asp + Phe183Gln + Thr207Ser + Leu211Thr
    + Gly213Asp
Gly 95Asp + Gly 98Pro + Ser 99Asp + Gly100Ser + Tyr102Glu
    + Ser103Glu
Gln 57Asp + Asp 58Glu + Thr 64Asp + Ser 97Glu + Gln200Ser
    + Tyr208Thr
Gly125Asp + Ser130Asp + Asn153Glu + Ala156Thr + Ser160Glu
    + Asn198Ser
Gly 59Asn + Leu124Thr + Ile159Met + Ala209Asp + Leu211Cys
    + Asn212Asp
Gly 95Gln + Gly100Asn + Ile105Met + Ala181Gln + Gln200Glu
    + Thr207Glu
Ile105Met + Ser126Asp + Asn153Glu + Gly205Gln + Thr207Pro
    + Leu211Met
Pro129Gly + Ala131His + Val197Thr + Thr207Asn + Ser210Glu
    + Gly213Glu
Gly 98Asp + Gly152Glu + Asn153Glu + Ser154Asp + Val197Ser
    + Gln200Asn
Asp 58Glu + Gly 61Pro + Val 93Gly + Ser 97Glu + Pro129Asn
    + Tyr161Ile
Ser130Asp + Gly152Ser + Val197Gly + Asn198Glu + Ser210Glu
    + Leu211Asp
Tyr102Val + Gly155Asn + Ser162Asp + Val197Asp + Gln200Asp
    + Ser210Asp
Thr 64Gly + Ser128Glu + Gln200Glu + Thr207Ser + Ser210Asp
    + Leu211Asp
Gln 57Glu + Tyr102Ser + Thr207Pro + Ser210Asp + Leu211Asp
    + Asn212Asp
Pro127Asn + Gly152Glu + Ile159Asn + Asn198Asp + Gln200Asn
    + Gly213Glu
Asn153Ser + Gly155Ser + Ile159Thr + Asn198Asp + Ser210Glu
    + Thr214Asp
Val 93Thr + Ser154Asp + Ser158Glu + Ser182Glu + Asn198Ser
    + Thr207Ser
Ser101Asp + Gly155Pro + Asn198Glu + Gln200Glu + Ala209Asn
    + Ser210Asp

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        Ser 99Glu + Ser126Asp + Pro127Asp + Ile159Ser + Gln200Ser
          + Leu211Met
        Pro129Asn + Asn153Asp + Ser158Asp + Gln200Ser + Pro204Ser
          + Gly205Ser
 5      Val197Cys + Gly205Asp + Thr207Asp + Ser210Asp + Leu211Ile
          + Asn212Ser
        Gly 61Asn + Asn198Asp + Gln200Glu + Thr207Glu + Tyr208Cys
          + Leu211His
        Asn 60Asp + Pro127Gln + Asn153Glu + Ser154Glu + Ala156Glu
10        + Thr207Pro
        Ser 99Glu + Asn153Asp + Ser154Asp + Ala156Glu + Gln185Asn
          + Pro195Ser
        Thr 64Asn + Ala156Asn + Val197Asp + Gln200Glu + Leu211Pro
          + Thr214Glu
15      Ser126Asp + Pro127Gln + Ser128Asp + Ser160Asp + Gln200Glu
          + Thr214Ser
        Asn153Asp + Gln185Glu + Asn198Ser + Gln200Glu + Leu211Cys
          + Gly213Asp
        Ile105Gln + Leu124Glu + Gly125Glu + Ser160Asp + Ala194Gln
20        + Pro204Glu
        Asp 58Glu + Leu 94Pro + Ser154Glu + Gly155Asp + Ser182Asp
          + Asn198Gln
        Ser 99Glu + Val197Glu + Thr207Gly + Ala209Asn + Ser210Glu
          + Leu211Asp
25      Gln 57Asn + Gly125Glu + Asn153Glu + Gln200Asn + Leu211Ser
          + Asn212Asp
        Leu 94Ser + Gly125Gln + Ser126Glu + Val197Asp + Ser210Asp
          + Asn212Glu
        Thr 64Glu + Ile159His + Asn198Glu + Gln200Asp + Leu211Cys
30        + Thr214Gly
        Gly 98Asp + Gly152Glu + Gly155Glu + Ala156Asp + Tyr208Met
          + Asn212Ser
        Gly 98Pro + Ser104Glu + Asn198Glu + Gln200Asp + Gly205Gln
          + Asn212Asp
35      Asp 58Glu + Thr 64Glu + Ala 96Ser + Tyr102His + Gln200Glu
          + Leu211Gly
        Leu 94Ala + Gly100Gln + Phe183Asp + Val193Gln + Val197Asp
          + Gln200Glu
        Asn 60Gln + Thr 64Gly + Gly125Glu + Ser154Glu + Gly157Asp
40        + Thr207Gly
        Val 93Met + Gly100Asp + Ser126Glu + Pro129Asp + Ala181Asn
          + Thr214Ser
        Gly125Asn + Ser182Glu + Phe183Tyr + Gln185Asp + Asn198Asp
          + Asn212Glu
45      Leu 94Gln + Gly125Asp + Ser128Glu + Pro129Glu + Asn198Gln
          + Ser210Glu
        Gly 59Gln + Thr 64Asp + Asn198Gln + Gln200Asn + Ser210Asp
          + Asn212Asp
        Gly 59Gln + Gly125Glu + Asn198Asp + Gln200Asp + Leu211Ile
50        + Gly213Asp
        Thr 64Asn + Gly125Glu + Asn153Asp + Gln185Glu + Thr207Ser
          + Leu211Asp
        Asp 58Glu + Gly 95Glu + Gly 98Glu + Gln185Asn + Pro204Ser
          + Ser210Asp
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Gln 57Glu + Gly 59Glu + Thr 64Asp + Gly152Ser + Ala156Glu
    + Leu211Ser
Asn 60Ser + Ala156Gln + Asn198Glu + Gly205Gln + Thr207Asp
    + Leu211Asp
5   Leu 94Ala + Ser101Asp + Ser154Glu + Ser156Asp + Ser160Asp
    + Leu211Thr
Asp 58Glu + Gln200Glu + Thr207Glu + Ser210Glu + Leu211Thr
    + Gly213Pro
Gly 61Gln + Gly152Asp + Gln200Glu + Ser210Glu + Leu211Gly
10   + Thr214Asp
Asn 60Glu + Tyr102Ile + Gln200Glu + Thr207Asp + Ser210Asp
    + Asn212Ser
Gly 59Ser + Val 93Asp + Ile159Asp + Ser160Glu + Thr207Asn
    + Gly213Ser
15 Thr 64Asn + Ser104Asp + Ile105Cys + Ala156Gly + Ser210Asp
    + Leu211Asp
Gln 57Asn + Gly 98Asp + Gly100Gln + Ser206Glu + Thr207Glu
    + Tyr206Met
Gln 57Asn + Ser158Asp + Ile159Glu + Phe183Met + Asn198Asp
20   + Thr207Asn
Gly 95Asp + Ala 96Gly + Ala131Gln + Thr207Glu + Tyr208Glu
    + Leu211Thr
Ser103Asp + Ser104Asp + Gln200Asn + Leu211Asn + Asn212Asp
    + Gly213Asp
25 Gly 61Gln + Gly 98Glu + Gly155Asp + Phe183Asp + Thr207Gln
    + Leu211Pro
Leu 94Ser + Gly 95Ser + Ser 97Glu + Gly 98Glu + Ala131Glu
    + Asn212Ser
Thr 64Asn + Tyr102Ile + Ser103Asp + Ser104Asp + Ala181Asn
30   + Tyr208Asp
Thr 64Gly + Tyr102Asp + Gln200Glu + Thr207Asn + Tyr208Gln
    + Ser210Glu
Ser 99Glu + Tyr102His + Ile159Ala + Gln200Asp + Thr207Gln
    + Ser210Glu
35 Thr 64Glu + Leu124Cys + Val197Glu + Gln200Asn + Ser210Asp
    + Leu211Gly
Thr 64Gly + Ser154Asp + Ala181Asn + Gln200Glu + Thr207Glu
    + Ser210Asp
Tyr102Ala + Ala181Asn + Ser182Glu + Gln200Asp + Thr207Asp
40   + Ser210Asp
Gly125Pro + Gly152Asn + Ser158Glu + Ile159Glu + Asn198Asp
    + Ser210Glu
Gln 57Ser + Asn 60Ser + Val 93Gln + Leu 94Gly + Gln200Glu
    + Gly213Asp
45 Gly 61Glu + Thr 64Ser + Val 93Asp + Asn198Ser + Pro204Glu
    + Tyr208Gly
Ser101Asp + Ser103Glu + Leu124Met + Gly155Ser + Ser210Asp
    + Leu211Gln
Ser 99Glu + Pro127Asp + Pro129Ser + Ser154Glu + Ser158Glu
50   + Thr207Ser
Gly 61Glu + Ser101Glu + Ser104Glu + Ile159Leu + Gly196Asn
    + Asn198Gln
Gly 59Asp + Asn 60Glu + Ser 99Glu + Val197Thr + Thr207Asp
    + Gly213Gln

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Gly 61Asn + Ala 96Glu + Gly 98Glu + Leu124Gln + Pro127Ser
    + Gly152Glu
Gly125Ser + Phe183Asp + Gln185Glu + Ala209Glu + Ser210Asp
    + Leu211His
Ser130Asp + Ile159Gly + Asn198Glu + Thr207Gln + Ser210Asp
    + Leu211Met
Pro127Gly + Ser130Asp + Tyr161Thr + Asn198Glu + Ser210Glu
    + Thr214Gln
Gly 98Pro + Tyr102Glu + Phe183Met + Asn198Asp + Gln200Asn
    + Ser210Asp
Ser 99Glu + Pro127Ser + Gly155Pro + Val197Cys + Asn198Glu
    + Leu211Glu
Leu 94Ser + Ser101Glu + Ile105Glu + Pro195Gln + Val197Glu
    + Asn198Glu
Pro127Glu + Asn198Ser + Pro204Glu + Gly205Ser + Ser206Asp
    + Thr207Pro
Gln 57Ser + Asn 60Asp + Gln185Asn + Asn198Glu + Leu211Thr
    + Asn212Asp
Asp 58Glu + Asn 60Asp + Tyr102Ala + Ile159Ser + Ser160Glu
    + Gly205Pro
Ala 96Gly + Ala181Asp + Asn198Asp + Thr207Glu + Ala209His
    + Ser210Glu
Gln 57Asn + Asn 60Glu + Gly 95Glu + Gly100Glu + Gly125Ser
    + Ser210Glu
Asp 58Glu + Phe183Ser + Pro204Glu + Thr207Ser + Ser210Glu
    + Asn212Glu
Leu 94Asp + Pro129Glu + Ala156Ser + Tyr161Asp + Gln185Ser
    + Asn212Ser
Gly 95Glu + Ser 99Asp + Ala156His + Val197Glu + Asn198Gln
    + Thr207Asn
Thr 64Glu + Pro127Asn + Ala156Glu + Ala209Asp + Ser210Asp
    + Leu211Asn
Gly 59Ser + Gly 63Pro + Pro129Ser + Gly157Glu + Gln185Asp
    + Ser210Glu
Gln 57Ser + Gly 59Asp + Leu 94Glu + Gly125Asp + Ser160Glu
    + Gln185Asn
Ile105Gln + Ser126Glu + Ser128Asp + Leu211Glu + Gly213Asp
    + Thr214Gly
Pro127Ser + Ser182Glu + Gln185Glu + Asn198Asp + Thr207Pro
    + Ser210Glu
Ala 96Asn + Ala131Pro + Ala181Asp + Thr207Asp + Ser210Asp
    + Leu211Asp
Asp 58Glu + Gly 59Pro + Ser126Asp + Gly152Asp + Ser158Glu
    + Leu211Ile
Gly155Asp + Ser182Asp + Val197Gly + Gln200Asp + Thr202Asn
    + Leu211Glu
Ser126Asp + Ile159Ser + Asn198Ser + Gln200Ser + Pro204Glu
    + Thr207Asp
Asn 60Ser + Gly 63Asn + Gln185Glu + Pro195Ser + Asn198Asp
    + Gln200Glu
Gly 59Ser + Ile105Asp + Ala181Asn + Asn198Asp + Gln200Glu
    + Leu211Thr
Leu 94Glu + Ser 99Asp + Gly100Gln + Ser103Asp + Gln200Glu
    + Tyr208Met

```
    Asp 58Glu + Leu 94Asp + Ser 99Glu + Gly100Pro + Gly157Asp
        + Tyr203Val
    Asp 58Glu + Thr 64Gln + Gly 98Glu + Ser 99Asp + Pro129Glu
        + Gly155Ser
 5  Val 93Gln + Ser101Asp + Ile105Asp + Asn198Asp + Gly205Ser
        + Leu211Asn
    Ser 99Glu + Ile105Ala + Ser210Glu + Leu211His + Asn212Glu
        + Thr214Asp
    Val 93Ala + Leu124His + Ala131Gly + Gly157Asp + Val197Asp
10      + Gly213Asp
    Ile105His + Gly152Ser + Ala156Asn + Ser158Glu + Val197Asp
        + Gly213Asp
    Gly 61Asp + Leu 94Thr + Val197Met + Asn198Gln + Gln200Glu
        + Gly205Glu
15  Tyr102Pro + Ser128Asp + Ser130Glu + Ala131Thr + Gln200Asp
        + Leu211Asp
    Gly 98Ser + Ser126Asp + Ser128Glu + Gly152Gln + Ser158Asp
        + Gly205Glu
    Ser130Asp + Phe183Ile + Thr207Gly + Tyr208Asp + Ser210Glu
20      + Asn212Asp
    Asp 58Glu + Leu 94Thr + Gly100Gln + Tyr102His + Ser206Asp
        + Thr214Pro
    Asp 58Glu + Thr 64Glu + Ser126Glu + Pro129Ser + Ala181Gly
        + Asn198Gln
25  Asn 60Ser + Leu 94Asp + Ala 96Glu + Ala181Glu + Gln165Asn
        + Thr207Gly
    Pro127Asp + Pro129Glu + Ala181Gln + Pro195Ser + Ser206Asp
        + Thr214Gln
    Gly152Gln + Ala156Asp + Thr207Asp + Ala209Glu + Leu211Pro
30      + Gly213Pro
    Ser103Glu + Gly157Ser + Ser158Glu + Ser160Glu + Tyr161His
        + Pro204Ser
    Leu 94Asp + Gly 95Gln + Gly 98Asp + Ser126Glu + Ser154Asp
        + Thr207Ser
35  Ser 97Glu + Ile105Glu + Leu124Asp + Phe183Gly + Thr207Gly
        + Leu211Gln
    Gly100Glu + Pro129Ser + Ala131Ser + Tyr161Val + Ser210Asp
        + Asn212Glu
    Gly 59Ser + Ser101Glu + Ile105Gln + Ala131His + Ser210Glu
40      + Asn212Glu
    Gln 57Asn + Pro127Gln + Ala156Asp + Ile159Asp + Pro195Gly
        + Ser206Asp
    Val 93His + Gly100Glu + Ser126Asp + Ser128Glu + Gln185Asp
        + Pro204Gly
45  Gly 95Ser + Ser 99Asp + Ser101Asp + Val197Asn + Leu211Met
        + Gly213Glu
    Gln 57Ser + Gly 61Glu + Ala 96Glu + Asn153Asp + Gly213Pro
        + Thr214Glu
    Gln 57Ser + Ala156Asp + Ser160Asp + Val197Gly + Leu211Asp
50      + Gly213Asp
    Gly 98Pro + Ser104Asp + Ala181Glu + Ser182Glu + Asn198Glu
        + Asn212Gln
    Ile105Glu + Leu124Thr + Val197Ser + Pro204Glu + Thr207Asp
        + Ser210Asp
```

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Asn 60Ser + Gly 61Pro + Ser160Asp + Gln185Asp + Gln200Asn
    + Ser210Glu

Gly 61Ser + Leu 94Asp + Ser104Asp + Leu124Asn + Asn198Ser
    + Gly213Asp

5 Asn 60Glu + Val 93Met + Gly 95Asp + Gly 98Asn + Asn198Glu
    + Leu211Cys

Gly 95Ser + Gly 98Asp + Ser 99Glu + Ser126Glu + Ser182Glu
    + Thr207Gly

Asp 58Glu + Gly 98Asp + Leu124Cys + Gln200Asp + Ser210Asp
10   + Asn212Ser

Leu 94Glu + Ser 97Asp + Ser158Glu + Val197Thr + Tyr208Ser
    + Leu211Ser

Ser101Glu + Ile105Glu + Ser154Glu + Gly157Glu + Asn198Ser
    + Leu211Ala

15 Gly 63Ser + Gly 98Glu + Gly100Pro + Gly152Asp + Gln200Asn
    + Gly213Asp

Leu 94Glu + Ser101Asp + Phe183Ala + Gln200Asn + Ser206Glu
    + Leu211Met

Gln 57Glu + Asp 58Glu + Leu124Pro + Asn198Ser + Ser206Asp
20   + Gly213Asp

Gly125Glu + Ala131His + Gly205Asp + Ala209Glu + Ser210Glu
    + Thr214Ser

Gly 59Ser + Gly100Glu + Ser103Asp + Asn198Ser + Tyr208Leu
    + Ser210Glu

25 Asp 58Glu + Asn 60Asp + Gly155Asp + Val197Asn + Thr207Ser
    + Thr214Ser

Ser 99Asp + Ser128Glu + Pro129Asp + Gly152Glu + Gln200Asn
    + Thr207Asn

Pro127Gln + Asn153Glu + Ser182Asp + Val197Cys + Ser210Glu
30   + Leu211Pro

Ser104Glu + Leu124Pro + Asn153Glu + Ala156Thr + Ile159Leu
    + Ser182Glu

Thr 64Asn + Tyr102Cys + Ser128Asp + Ser158Asp + Phe183Glu
    + Asn198Ser

35 Ala 96Gln + Tyr161Asn + Gln185Asn + Val197Glu + Gly205Glu
    + Ser210Asp

Gly 98Ser + Ser101Glu + Pro127Gly + Gln200Asp + Leu211Asp
    + Thr214Glu

Gly 59Gln + Thr 64Glu + Gly 95Ser + Gly155Glu + Ser158Asp
40   + Val197Cys

Tyr102Val + Ser103Glu + Asn198Asp + Gln200Glu + Thr207Glu
    + Ala209Asn

Asn 60Glu + Gly 98Gln + Asn153Glu + Ser210Asp + Leu211Ala
    + Gly213Glu

45 Asp 58Glu + Gly 61Glu + Ser101Glu + Leu124Asn + Pro129Gly
    + Ser130Asp

Ser126Glu + Ser130Asp + Ala131Thr + Gln200Asn + Thr202Gln
    + Pro204Asp

Gly 63Gln + Ser 97Glu + Ser126Asp + Ser130Asp + Asn198Ser
50   + Gln200Ser

Val 93Gln + Leu 94Cys + Ser 97Glu + Ser101Glu + Asn212Gln
    + Gly213Ser

Asp 58Glu + Gly 95Glu + Ser154Asp + Ser158Asp + Thr207Pro
    + Tyr208Val

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Asn 60Gln + Thr 64Ser + Pro129Asn + Pro204Asp + Ser210Asp
     + Leu211Ile
Ser 99Asp + Ile159Gln + Val197Gly + Asn198Glu + Leu211Val
     + Gly213Asp
Ser 97Glu + Gly100Glu + Gly152Glu + Gly155Gln + Ala181Ser
     + Ala209His
Asp 58Glu + Ala 96Glu + Ser126Glu + Asn153Asp + Phe183Leu
     + Gly205Asn
Gly 98Asp + Pro127Glu + Gly152Pro + Ser158Glu + Gly205Pro
     + Leu211Gln
Ser154Glu + Gly157Asp + Ile159Asn + Asn198Glu + Gln200Asn
     + Tyr203Asn
Gly 63Ser + Phe183Glu + Thr207Glu + Ser210Glu + Leu211Pro
     + Thr214Asp
Gly100Glu + Asn153Ser + Ser154Asp + Ser160Glu + Asn198Ser
     + Gly213Glu
Gln 57Glu + Ala 96His + Gly 98Pro + Ser160Asp + Pro204Asp
     + Tyr208Gly
Gly 59Asp + Gly 95Pro + Ala194Gln + Asn198Asp + Gln200Asn
     + Thr214Asp
Gly 95Glu + Val197Glu + Asn198Gln + Gln200Glu + Thr207Gln
     + Leu211Ala
Gly 98Asp + Ser128Asp + Gly155Pro + Ser158Asp + Gln185Glu
     + Gln200Ser
Gly 98Glu + Leu124Asp + Gly125Asn + Ile159Gly + Val197Asp
     + Leu211Ala
Thr 64Asp + Gly152Asn + Ser160Glu + Gln185Asn + Thr207Asp
     + Ser210Asp
Thr 64Asp + Gly155Asp + Ala156Ser + Val197Ser + Asn198Glu
     + Leu211Asp
Gln 57Asn + Thr 64Pro + Ile105Glu + Ser126Glu + Ser160Asp
     + Ser206Glu
Gly 98Asn + Gly157Asp + Phe183Glu + Val197Asn + Ser206Asn
     + Thr214Glu
Ser 97Asp + Ser126Glu + Ser128Asp + Pro129Asn + Ala156Asp
     + Leu211Gln
Gln 57Glu + Leu124Pro + Phe183Asn + Val197Glu + Gly205Glu
     + Gly213Glu
Ser126Asp + Ser158Glu + Gln200Asp + Thr207Pro + Leu211Glu
     + Asn212Ser
Gly 98Ser + Gly100Pro + Pro129Glu + Gly157Glu + Ser158Glu
     + Asn198Asp
Gly 61Asp + Gln185Asp + Asn198Gln + Gln200Asp + Thr207Asp
     + Leu211Ser
Asp 58Glu + Asn198Gln + Gln200Glu + Thr207Glu + Leu211Ala
     + Asn212Glu
Gly 59Asp + Ser 97Glu + Pro129Asn + Gly152Pro + Ser182Glu
     + Thr214Asp
Gly 59Glu + Thr 64Glu + Pro127Gln + Pro129Asn + Ala131Gly
     + Asn153Glu
Pro127Glu + Ser130Asp + Ser154Glu + Ile159Cys + Ser210Glu
     + Leu211Ser
Asp 58Glu + Val 93Asn + Ser 97Asp + Gly100Glu + Gly152Pro
     + Asn198Glu

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Gly125Glu + Gly155Asp + Gly157Asn + Gln200Glu + Gly205Asn
    + Ser210Asp
Ala 96Ser + Ser103Glu + Leu124Asp + Gln200Glu + Ser210Glu
    + Leu211Cys
5 Ser101Asp + Pro129Asp + Gly155Asn + Ala181Glu + Ser182Asp
    + Gly205Pro
Asp 58Glu + Gly 61Glu + Ala 96His + Asn153Asp + Val197Gln
    + Gln200Glu
Asn 60Asp + Ser 99Glu + Gly155Pro + Ala181Glu + Val197Asn
10   + Asn212Asp
Asn 60Gln + Gly 98Asn + Ser130Asp + Ala181Asp + Ala209Asp
    + Asn212Asp
Gly 61Asp + Ala131Asp + Val197Ala + Asn198Asp + Gln200Asp
    + Thr214Asn
15 Thr 64Asp + Ser 99Asp + Ser101Glu + Ala181Gln + Gln200Ser
    + Ser210Glu
Leu124Ser + Tyr161Glu + Ser182Asp + Val197Glu + Thr207Gln
    + Leu211Asn
Gly 95Glu + Gly 98Asp + Ser103Asp + Ala181Gln + Thr207Gln
20   + Asn212Asp
Asn 60Glu + Ser 97Glu + Ser101Glu + Leu124Ala + Pro127Glu
    + Val197Gln
Val 93Asp + Ala 96Glu + Ala131Asp + Gly152Gln + Gly196Ser
    + Val197His
25 Val 93Gly + Ala131Asp + Ile159Gly + Ser182Glu + Asn198Gln
    + Gly213Asp
Ser130Asp + Ser182Asp + Gln185Ser + Val197Asn + Asn198Glu
    + Ser210Asp
Ile159Thr + Ser182Asp + Gln200Asp + Gly205Asp + Thr207Asn
30   + Asn212Glu
Val 93Thr + Ser104Glu + Pro127Ser + Ser128Asp + Ser130Glu
    + Asn153Glu
Asn 60Glu + Leu 94Asp + Gln200Ser + Gly205Asp + Ser210Asp
    + Leu211Gln
35 Gly 95Asp + Ser101Asp + Ser130Glu + Asn198Asp + Gln200Ser
    + Leu211Ala
Ser104Asp + Ser154Glu + Thr207Asn + Ala209Asp + Ser210Glu
    + Leu211Asn
Asp 58Glu + Gly 59Glu + Ile105Leu + Ser126Asp + Phe183Asn
40   + Ser210Asp
Gly 59Glu + Asn 60Asp + Gly 95Asn + Pro129Glu + Gly152Ser
    + Gln185Glu
Gly 59Asp + Ser160Glu + Gly196Ser + Gly205Ser + Ser210Asp
    + Leu211Asp
45 Asn 60Glu + Ser 99Asp + Gly157Glu + Phe183Leu + Val197Asn
    + Thr207Gln
Ser 99Glu + Asn198Asp + Ser206Glu + Thr207Asp + Tyr208Asn
    + Leu211His
Gly 98Glu + Ser 99Asp + Ala131Pro + Asn153Glu + Tyr161Pro
50   + Tyr208Asp
Ser 97Glu + Gly 98Glu + Ser182Asp + Thr207Gln + Ser210Asp
    + Gly213Ser
Ser 97Asp + Gly 98Asp + Ile105Val + Pro129Gly + Gln200Asp
    + Gly205Glu

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Asp 58Glu + Ser103Asp + Ser104Asp + Phe183Gly + Val197Pro
    + Asn198Asp
Asn 60Ser + Ser 99Glu + Gly100Asp + Pro129Gly + Gly205Asp
    + Ser210Glu
5 Ser 99Glu + Ser154Asp + Tyr161Cys + Val197Ser + Gln200Asp
    + Ser210Asp
Ser101Glu + Gly155Asp + Gln185Ser + Asn198Gln + Gln200Asp
    + Ser210Glu
Gly 59Asp + Tyr102Asn + Ser158Asp + Gln200Asp + Tyr208Val
10   + Ser210Glu
Gly 59Asp + Ala156Gln + Ile159Asp + Phe183Gly + Gln200Asp
    + Ser210Asp
Pro127Ser + Ser160Asp + Ser182Asp + Val197Gln + Gln200Glu
    + Ser210Asp
15 Gln 57Asn + Val 93Asp + Pro127Glu + Asn198Gln + Gln200Glu
    + Ser210Glu
Leu 94Glu + Pro129Gln + Ser154Asp + Val197His + Gln200Asp
    + Ser210Glu
Ala 96Ser + Gly 98Gln + Ser126Asp + Ser154Asp + Val197Thr
20   + Thr207Asp
Gln 57Ser + Ser104Asp + Gly125Asp + Ser160Glu + Gly205Pro
    + Ser210Glu
Leu 94Gln + Ser 99Asp + Gly155Pro + Val193Asn + Gln200Asp
    + Thr207Asp
25 Gly 95Glu + Gln200Glu + Thr202Ser + Thr207Asp + Leu211Gln
    + Thr214Gln
Thr 64Gln + Ala 96Thr + Ser104Asp + Gln200Asp + Thr207Asp
    + Thr214Pro
Thr 64Glu + Tyr102Cys + Ala156Ser + Phe183Glu + Pro195Gln
30   + Thr207Glu
Gly 98Glu + Val197His + Thr207Gln + Ala209Ser + Ser210Asp
    + Gly213Asp
Gly 95Ser + Gly157Asp + Phe183Glu + Thr207Asp + Ala209Gln
    + Ser210Asp
35 Asp 58Glu + Val 93Asp + Ser126Glu + Ala131Glu + Gly196Gln
    + Pro204Asn
Gln 57Ser + Pro127Glu + Gln185Asn + Asn198Asp + Gln200Glu
    + Ser206Glu
Val 93Glu + Gly152Asn + Thr207Glu + Ser210Asp + Leu211Ser
40   + Gly213Asp
Thr 64Gly + Ser 99Glu + Ser103Glu + Pro127Asn + Gly152Glu
    + Ser156Glu
Gly157Asn + Ser158Glu + Asn198Asp + Ser206Asp + Thr207Gln
    + Ser210Asp
45 Ala 96Asp + Ser126Glu + Ser160Glu + Tyr161Leu + Ser210Glu
    + Gly213Ser
Asp 58Glu + Ala 96Ser + Ser101Asp + Ser104Glu + Ala209Gln
    + Ser210Asp
Gly 61Asp + Thr 64Gln + Val 93Cys + Ile105Ser + Leu211Val
50   + Asn212Glu
Gly 61Glu + Phe183Ser + Tyr203Thr + Thr207Asn + Tyr208Gly
    + Asn212Glu
Ser 97Glu + Ile105Cys + Pro127Ser + Ser160Asp + Asn198Glu
    + Ser210Asp

Leu 94Asp + Ser130Glu + Asn198Asp + Pro204Asn + Thr207Gln
+ Ser210Asp
Ile105Asp + Leu124Pro + Ile159Leu + Asn198Glu + Ser206Asp
+ Leu211Glu
Tyr102Gly + Ala131Ser + Ser182Glu + Phe183Pro + Thr207Glu
+ Ser210Asp
Asn 60Gln + Gly 61Glu + Ser 97Asp + Gly125Glu + Tyr208Pro
+ Ser210Asp
Gly 98Gln + Ser160Glu + Ala181Thr + Asn198Asp + Ser206Asp
+ Asn212Glu
Leu124Ala + Ser128Glu + Asn153Gln + Gly205Asp + Thr207Glu
+ Asn212Glu
Gly 59Glu + Gly100Pro + Ser103Glu + Ile105Glu + Val197Gly
+ Ser210Glu
Asn 60Glu + Ser 97Glu + Pro127Asp + Val193Met + Thr207Asp
+ Ala209Asn
Thr 64Gln + Ala 96Asn + Ser101Glu + Ser130Asp + Asn198Ser
+ Leu211Asp
Gly 61Pro + Leu 94Glu + Ser103Asp + Ile159Pro + Leu211Asn
+ Asn212Glu
Val 93Cys + Leu 94Asp + Gly100Ser + Ser103Glu + Ser206Asp
+ Tyr208Gly
Ser130Asp + Gly152Glu + Ser154Glu + Gly157Ser + Ala181Pro
+ Asn198Glu
Gly 59Glu + Ser130Asp + Ser154Asp + Gln185Asp + Thr207Gly
+ Leu211Pro
Gln 57Glu + Leu 94Ala + Ser101Asp + Ser126Glu + Ala131Gln
+ Ser154Asp
Ser103Asp + Ile105Met + Gly125Asn + Ser160Asp + Val197Ser
+ Thr214Asp
Asp 58Glu + Val 93Asp + Gly125Ser + Gly152Ser + Val193His
+ Gln200Glu
Asp 58Glu + Gly 98Asp + Leu124Ser + Gly125Glu + Tyr161Met
+ Gln200Glu
Gly 61Asp + Leu 94Ile + Asn153Asp + Ser158Asp + Val197Ala
+ Ser210Glu
Asn 60Ser + Val 93Ser + Ser103Glu + Ser160Glu + Asn198Asp
+ Gln200Asp
Asn 60Gln + Ser 97Asp + Ser126Asp + Asn198Glu + Gln200Asp
+ Ala209Gly
Val 93Ser + Tyr102Asn + Ser104Asp + Gly152Asp + Asn198Glu
+ Gln200Glu
Gly 59Asp + Ile105Met + Ser158Glu + Ile159Ser + Asn198Glu
+ Gln200Asp
Gly 59Glu + Ser101Glu + Ile159Cys + Asn198Glu + Val199Gln
+ Gln200Asp
Gly 61Asp + Pro127Asp + Gln185Asp + Asn198Ser + Gln200Ser
+ Ser210Asp
Gly100Glu + Ser104Glu + Ser182Glu + Thr207Gly + Tyr208Met
+ Ser210Glu
Thr 64Gly + Gly157Asp + Ser182Glu + Val197Gln + Asn198Ser
+ Ser210Asp
Ser128Glu + Ser130Asp + Tyr161Val + Asn198Glu + Ser206Glu
+ Gly213Asn

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Gly 59Gln + Thr 64Asp + Ser 99Glu + Asn198Asp + Pro204Glu
    + Thr207Asn
Gly 95Glu + Ser126Asp + Gln185Ser + Val197Cys + Gln200Glu
    + Leu211Glu
Gly 98Pro + Ser 99Glu + Ser158Asp + Gln200Asp + Ala209Ser
    + Leu211Asp
Ser101Glu + Pro129Glu + Asn153Ser + Ser160Asp + Thr207Glu
    + Leu211Cys
Asn 60Glu + Gly 98Asp + Pro127Asp + Ala131Gln + Ala161Asp
    + Asn198Ser
Asp 58Glu + Ser126Asp + Gly157Asn + Thr207Ser + Leu211Asp
    + Gly213Asp
Ser104Asp + Leu124Gly + Asn153Asp + Ser182Glu + Asn198Glu
    + Thr207Asn
Gly 95Ser + Ser103Glu + Pro129Glu + Ser160Asp + Gln200Asp
    + Leu211Pro
Gly 59Glu + Gly125Asp + Pro127Glu + Ala131His + Ala209Asp
    + Leu211Ile
Pro127Glu + Gly157Asn + Pro204Gly + Thr207Asp + Ala209Asp
    + Thr214Asp
Gln 57Glu + Leu 94Glu + Ser103Asp + Gly152Asp + Gly205Ser
    + Leu211Ala
Ala 96Glu + Ser103Asp + Gly152Gln + Ser158Glu + Ser160Asp
    + Thr214Asn
Gly 61Glu + Tyr102His + Asn153Asp + Phe183His + Gln185Asn
    + Ser210Asp
Ser 97Asp + Ser126Asp + Pro129Glu + Val197His + Asn198Asp
    + Gln200Asn
Asn 60Glu + Ala 96Pro + Ile105Asp + Leu124Ala + Ser130Glu
    + Ile159Ser
Gln 57Glu + Pro127Gly + Ser128Glu + Ala131Ser + Ser210Asp
    + Asn212Glu
Ala 96Glu + Tyr102Asp + Ser128Glu + Ala156Pro + Tyr161Val
    + Leu211Thr
Gln 57Ser + Asp 58Glu + Ser 99Asp + Gln200Glu + Tyr208Ser
    + Asn212Glu
Gly 59Asp + Leu 94Met + Ile105Ala + Gly152Glu + Ser182Glu
    + Gln185Ser
Asn 60Glu + Ala156Gly + Val197Asp + Asn198Ser + Gln200Glu
    + Ser206Asp
Gly100Ser + Ser101Asp + Gly125Gln + Ser126Asp + Pro127Asn
    + Asn198Glu
Ala 96Glu + Ser 99Asp + Ser160Asp + Ala161Gly + Gly205Ser
    + Ser210Asp
Leu124Asp + Gly125Pro + Ser130Glu + Ser154Asp + Gly205Gln
    + Ala209Thr
Ser101Glu + Gly152Glu + Gly155Glu + Asn198Gln + Gln200Asp
    + Asn212Ser
Gly 95Asn + Ser103Asp + Gly152Glu + Gly155Asp + Tyr208Val
    + Ser210Glu
Ser 99Glu + Gly100Ser + Gly125Asn + Gly152Asp + Gly155Asp
    + Gly205Asp
Ala 96Glu + Ser160Asp + Tyr161Met + Phe183Leu + Gln185Asp
    + Gly205Asp

Leu 94Glu + Ser104Asp + Gly157Glu + Asn198Gln + Thr207Asn
  + Ala209Asp
Thr 64Gln + Ser101Glu + Ile105Pro + Gly125Ser + Pro129Glu
  + Thr214Asn
Gly 98Gln + Tyr102Gln + Gly125Asn + Ser126Glu + Ser158Glu
  + Asn198Glu
Leu 94Asp + Tyr161Ile + Ala181Gly + Phe183Asp + Gln200Glu
  + Thr214Asp

TABLE 38
Preferred Subtilisin 309 Variants

--Single Mutation--
Thr207Glu
Ser210Glu
Ser210Asp
Ser210Gly
Val197Glu

--Double Mutation--
Gln200Glu + Ser210Glu
Val199Leu + Ser210Glu
Val199Leu + Ser210Asp
Pro204Ala + Ala209Thr
Thr207Glu + Ser210Glu
Tyr208Phe + Leu211Asn
Ala194Glu + Ser210Glu
Gln200Glu + Tyr211Asn
Gln206Glu + Thr207Glu

--Triple Mutation--
Gln200Pro + Gly205Ala + Ser210Glu
Thr207Glu + Ser210Glu + leu211Asn
Val199Ile + Pro204Asn + Thr207Glu
Gln200Glu + Ser210Glu + Leu211Asp
Gln200Glu + Thr207Glu + Leu211Glu
Gln200Glu + Thr207Gln + Ser210Glu

--Quadruple Mutation--
Pro204Ala + Thr207Glu + Ser210Glu + Leu211Asn
Gln206Glu + Thr207Glu + Ser210Glu + Leu211Gly

--Quintuple Mutation--
Val197Leu + Pro204Ala + Thr207Glu + Ser210Glu + Leu217Asp
Asn198Glu + Gln200Glu + Thr207Glu + Ser210Glu + Leu211Asp

II.   Cleaning Compositions

In another embodiment of the present invention, an effective amount of
one or more enzyme variants of the present invention are included in
compositions useful for cleaning a variety of surfaces in need of proteinaceous

stain removal. Such cleaning compositions include detergent compositions for cleaning hard surfaces, unlimited in form (e.g., liquid and granular); detergent compositions for cleaning fabrics, unlimited in form (e.g., granular, liquid and bar formulations); dishwashing compositions (unlimited in form); oral cleaning compositions, unlimited in form (e.g., dentifrice, toothpaste and mouthwash formulations), denture cleaning compositions, unlimited in form (e.g., liquid, tablet); and contact lens cleaning compositions, unlimited in form (e.g., liquid, tablet). As used herein, "effective amount of enzyme variant" refers to the quantity of enzyme variant necessary to achieve the enzymatic activity necessary in the specific cleaning composition. Such effective amounts are readily ascertained by one of ordinary skill in the art and is based on many factors, such as the particular enzyme variant used, the cleaning application, the specific composition of the cleaning composition, and whether a liquid or dry (e.g., granular, bar) composition is required, and the like. Preferably the cleaning compositions of the present invention comprise from about 0.0001% to about 10% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 1%, more preferably still from about 0.01% to about 0.1%. Several examples of various cleaning compositions wherein the enzyme variants of the present invention may be employed are discussed in further detail below. All parts, percentages and ratios used herein are by weight unless otherwise specified.

As used herein, "non-fabric cleaning compositions" include hard surface cleaning compositions, dishwashing compositions, oral cleaning compositions, denture cleaning compositions and contact lens cleaning compositions.

A.   Cleaning Compositions for Hard Surfaces, Dishes and Fabrics

The enzymes of the present invention can be used in any detergent composition where high sudsing and good insoluble substrate removal are desired. Thus the enzyme variants of the present invention can be used with various conventional ingredients to provide fully-formulated hard-surface cleaners, dishwashing compositions, fabric laundering compositions and the like. Such compositions can be in the form of liquids, granules, bars and the like. Such compositions can be formulated as modern "concentrated" detergents which contain as much as 30%-60% by weight of surfactants.

The cleaning compositions herein can optionally, and preferably, contain various anionic, nonionic, zwitterionic, etc., surfactants. Such surfactants are typically present at levels of from about 5% to about 35% of the compositions.

Nonlimiting examples of surfactants useful herein include the

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conventional C_{11}-C_{18} alkyl benzene sulfonates and primary and random alkyl sulfates, the C_{10}-C_{18} secondary (2,3) alkyl sulfates of the formulas $CH_3(CH_2)x(CHOSO_3^-M^+)CH_3$ and $CH_3(CH_2)y(CHOSO_3^-M^+)$ CH_2CH_3 wherein x and (y+1) are integers of at least about 7, preferably at least about 9, and M is a water-solubilizing cation, especially sodium, the C_{10}-C_{18} alkyl alkoxy sulfates (especially EO 1-5 ethoxy sulfates), C_{10}-C_{18} alkyl alkoxy carboxylates (especially the EO 1-5 ethoxycarboxylates), the C_{10}-C_{18} alkyl polyglycosides, and their corresponding sulfated polyglycosides, C_{12}-C_{18} alpha-sulfonated fatty acid esters, C_{12}-C_{18} alkyl and alkyl phenol alkoxylates (especially ethoxylates and mixed ethoxy/propoxy), C_{12}-C_{18} betaines and sulfobetaines ("sultaines"), C_{10}-C_{18} amine oxides, and the like. The alkyl alkoxy sulfates (AES) and alkyl alkoxy carboxylates (AEC) are preferred herein. {Use of such surfactants in combination with the aforesaid amine oxide and/or betaine or sultaine surfactants is also preferred, depending on the desires of the formulator.} Other conventional useful surfactants are listed in standard texts. Particularly useful surfactants include the C_{10}-C_{18} N-methyl glucamides disclosed in US Patent 5, 194,639, Connor et al., issued March 16, 1993, incorporated herein by reference.

A wide variety of other ingredients useful in detergent cleaning compositions can be included in the compositions herein, including other active ingredients, carriers, hydrotropes, processing aids, dyes or pigments, solvents for liquid formulations, etc. If an additional increment of sudsing is desired, suds boosters such as the C_{10}-C_{16} alkolamides can be incorporated into the compositions, typically at about 1% to about 10% levels. The C_{10}-C_{14} monoethanol and diethanol amides illustrate a typical class of such suds boosters. Use of such suds boosters with high sudsing adjunct surfactants such as the amine oxides, betaines and sultaines noted above is also advantageous. If desired, soluble magnesium salts such as $MgCl_2$, $MgSO_4$, and the like, can be added at levels of, typically, from about 0.1% to about 2%, to provide additionally sudsing.

The liquid detergent compositions herein can contain water and other solvents as carriers. Low molecular weight primary or secondary alcohols exemplified by methanol, ethanol, propanol, and isopropanol are suitable. Monohydric alcohols are preferred for solubilizing surfactants, but polyols such as those containing from about 2 to about 6 carbon atoms and from about 2 to about 6 hydroxy groups (e.g., 1,3-propanediol, ethylene glycol, glycerine, and 1,2-propanediol) can also be used. The compositions may contain from about 5% to about 90%, typically from about 10% to about 50% of such carriers.

The detergent compositions herein will preferably be formulated such that during use in aqueous cleaning operations, the wash water will have a pH between about 6.8 and about 11.0. Finished products thus are typically formulated at this range. Techniques for controlling pH at recommended usage levels include the use of buffers, alkalis, acids, etc., and are well known to those skilled in the art.

When formulating the hard surface cleaning compositions and fabric cleaning compositions of the present invention, the formulator may wish to employ various builders at levels from about 5% to about 50% by weight. Typical builders include the 1-10 micron zeolites, polycarboxylates such as citrate and oxydisuccinates, layered silicates, phosphates, and the like. Other conventional builders are listed in standard formularies.

Likewise, the formulator may wish to employ various additional enzymes, such as cellulases, lipases, amylases and proteases in such compositions, typically at levels of from about 0.001% to about 1% by weight. Various detersive and fabric care enzymes are well-known in the laundry detergent art.

Various bleaching compounds, such as the percarbonates, perborates and the like, can be used in such compositions, typically at levels from about 1% to about 15% by weight. If desired, such compositions can also contain bleach activators such as tetraacetyl ethylenediamine, nonanoyloxybenzene sulfonate, and the like, which are also known in the art. Usage levels typically range from about 1% to about 10% by weight.

Various soil release agents, especially of the anionic oligoester type, various chelating agents, especially the aminophosphonates and ethylenediaminedisuccinates, various clay soil removal agents, especially ethoxylated tetraethylene pentamine, various dispersing agents, especially polyacrylates and polyasparatates, various brighteners, especially anionic brighteners, various suds suppressors, especially silicones and secondary alcohols, various fabric softeners, especially smectite clays, and the like can all be used in such compositions at levels ranging from about 1% to about 35% by weight. Standard formularies and published patents contain multiple, detailed descriptions of such conventional materials.

Enzyme stabilizers may also be used in the cleaning compositions of the present invention. Such enzyme stabilizers include propylene glycol (preferably from about 1% to about 10%), sodium formate (preferably from about 0.1% to about 1%) and calcium formate (preferably from about 0.1% to about 1%).

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1.   Hard surface cleaning compositions

As used herein "hard surface cleaning composition" refers to liquid and granular detergent compositions for cleaning hard surfaces such as floors, walls, bathroom tile, and the like.   Hard surface cleaning compositions of the
5   present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about .01% to about 5%, more preferably still from about .05% to about 1% by weight of active enzyme of the composition. In addition to comprising one or more enzyme variants of the present invention, such hard
10   surface cleaning compositions typically comprise a surfactant and a water-soluble sequestering builder. In certain specialized products such as spray window cleaners, however, the surfactants are sometimes not used since they may produce a filmy/streaky residue on the glass surface.

The surfactant component, when present, may comprise as little as
15   0.1% of the compositions herein, but typically the compositions will contain from about 0.25% to about 10%, more preferably from about 1% to about 5% of surfactant.

Typically the compositions will contain from about 0.5% to about 50% of a detergency builder, preferably from about 1% to about 10%.   Preferably
20   the pH should be in the range of about 8 to 12.   Conventional pH adjustment agents such as sodium hydroxide, sodium carbonate or hydrochloric acid can be used if adjustment is necessary.

Solvents may be included in the compositions. Useful solvents include, but are not limited to, glycol ethers such as diethyleneglycol monohexyl ether,
25   diethyleneglycol   monobutyl   ether,   ethyleneglycol   monobutyl   ether, ethyleneglycol   monohexyl   ether,   propyleneglycol   monobutyl   ether, dipropyleneglycol monobutyl ether, and diols such as 2,2,4-trimethyl-1,3-pentanediol and 2-ethyl-1,3-hexanediol.   When used, such solvents are typically present at levels of from about 0.5% to about 15%, preferably from
30   about 3% to about 11%.

Additionally, highly volatile solvents such as isopropanol or ethanol can be used in the present compositions to facilitate faster evaporation of the composition from surfaces when the surface is not rinsed after "full strength" application of the composition to the surface. When used, volatile solvents are
35   typically present at levels of from about 2% to about 12% in the compositions.

The hard surface cleaning composition embodiment of the present invention is illustrated by the following examples.

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Examples 7-12

	Liquid Hard Surface Cleaning Compositions					
	Example No.					
Component	7	8	9	10	11	12
Thr207Glu	0.05	0.50	0.02	0.03	0.10	0.03
Val199Leu + Ser210Asp	--	--	--	--	0.20	0.02
Na_2DIDA*						
EDTA**	--	--	2.90	2.90	--	--
Na Citrate	--	--	--	--	2.90	2.90
NaC_{12} Alkyl-benzene sulfonate	1.95	--	1.95	--	1.95	--
NaC_{12} Alkylsulfate	--	2.20	--	2.20	--	2.20
NaC_{12}(ethoxy)*** sulfate	--	2.20	--	2.20	--	2.20
C_{12} Dimethylamine oxide	--	0.50	--	0.50	--	0.50
Na Cumene sulfonate	1.30	--	1.30	--	1.30	--
Hexyl Carbitol***	6.30	6.30	6.30	6.30	6.30	6.30
Water****	balance to 100%					

*Disodium N-diethyleneglycol-N,N-iminodiacetate

**Na_4 ethylenediamine diacetic acid

***Diethyleneglycol monohexyl ether

****All formulas adjusted to pH 7

        In Examples 7-10, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Thr207Glu, with substantially similar results.

        In Examples 11-12, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val199Leu + Ser210Asp, with substantially similar results.

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Examples 13-18
Spray Compositions for Cleaning Hard Surfaces
and Removing Household Mildew

Component	Example No.					
	13	14	15	16	17	18
Gln200Glu + Thr207Glu	0.50	0.05	0.60	0.30	0.20	0.30
Ser210Glu	-	-	-	-	0.30	0.10
Sodium octyl sulfate	2.00	2.00	2.00	2.00	2.00	2.00
Sodium dodecyl sulfate	4.00	4.00	4.00	4.00	4.00	4.00
Sodium hydroxide	0.80	0.80	0.80	0.80	0.80	0.80
Silicate (Na)	0.04	0.04	0.04	0.04	0.04	0.04
Perfume	0.35	0.35	0.35	0.35	0.35	0.35
Water	balance to 100%					

Product pH is about 7.

In Examples 13-16, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu, with substantially similar results.

In Examples 17-18, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu and Ser210Glu, with substantially similar results.

2.   Dishwashing Compositions

In another embodiment of the present invention, dishwashing compositions comprise one or more enzyme variants of the present invention. As used herein, "dishwashing composition" refers to all forms for compositions for cleaning dishes, including but not limited to, granular and liquid forms. The dishwashing composition embodiment of the present invention is illustrated by the following examples.

Examples 19-24

Dishwashing Composition

Component	Example No.					
	19	20	21	22	23	24
Gln200Pro + Gly205Ala + Ser210Asp	0.05	0.50	0.02	0.40	0.10	0.03
Val199Leu + Ser210Asp	-	-	-	-	0.40	0.02
C_{12}-C_{14} N-methyl-glucamide	0.90	0.90	0.90	0.90	0.90	0.90
C_{12} ethoxy (1) sulfate	12.00	12.00	12.00	12.00	12.00	12.00
2-methyl undecanoic acid	4.50	4.50	4.50	4.50	4.50	4.50
C_{12} ethoxy (2) carboxylate	4.50	4.50	4.50	4.50	4.50	4.50
C_{12} alcohol ethoxylate (4)	3.00	3.00	3.00	3.00	3.00	3.00
C_{12} amine oxide	3.00	3.00	3.00	3.00	3.00	3.00
Sodium cumene sulfonate	2.00	2.00	2.00	2.00	2.00	2.00
Ethanol	4.00	4.00	4.00	4.00	4.00	4.00
Mg^{++} (as $MgCl_2$)	0.20	0.20	0.20	0.20	0.20	0.20
Ca^{++} (as $CaCl_2$)	0.40	0.40	0.40	0.40	0.40	0.40
Water	balance to 100%					

Product pH is adjusted to 7.

In Examples 19-22, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Pro + Gly205Ala + Ser210Asp, with substantially similar results.

In Examples 23-24, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Pro + Gly205Ala + Ser210Asp and Val199Leu + Ser210Asp, with substantially similar results.

3.   Fabric cleaning compositions

In another embodiment of the present invention, fabric cleaning compositions comprise one or more enzyme variants of the present invention. As used herein, "fabric cleaning composition" refers to all forms for detergent compositions for cleaning fabrics, including but not limited to, granular, liquid and bar forms. Preferred fabric cleaning compositions are those in the liquid form.

a.   Granular fabric cleaning compositions

The granular fabric cleaning compositions of the present invention contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from

about 0.005% to about 5%, more preferably from about 0.01% to about 1% by weight of active enzyme of the composition. In addition to one or more enzyme variants, the granular fabric cleaning compositions typically comprise at least one surfactant, one or more builders, and, in some cases, a bleaching agent.

The granular fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 25-28
Granular Fabric Cleaning Composition

Component	Example No.			
	25	26	27	28
Ser99Asp	0.10	0.20	0.03	0.05
Ser99Gly	--	--	0.02	0.05
C_{13} linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylaenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

In Examples 25-26, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser99Asp, with substantially similar results.

In Examples 27-28, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser99Asp and Ser99Gly, with substantially similar results.

Examples 29-32
Granular Fabric Cleaning Composition

Component	Example No.			
	29	30	31	32
Gln200Glu + Thr207Glu + Ser210Glu	0.10	0.20	0.03	0.05
Asn74Asp + Pro204Ala + Thr207Glu	-	-	0.02	0.05
C_{12} alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C_{12}-C_{14} secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 29-30, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu + Ser210Glu, with substantially similar results.

In Examples 31-32, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Gln200Glu + Thr207Glu + Ser210Glu and Asn74Asp + Pro204Ala + Thr207Glu, with substantially similar results.

Examples 33-36
Granular Fabric Cleaning Composition

Component	Example No.			
	33	34	35	36
Leu 94Gly + Gln200Glu	0.10	0.20	0.03	0.05
Gln 57Ser + Leu 94Gly + Gln200Glu	-	-	0.02	0.05
C_{13} linear alkyl benzene sulfonate	22.00	22.00	22.00	22.00
Phosphate (as sodium tripolyphosphates)	23.00	23.00	23.00	23.00
Sodium carbonate	23.00	23.00	23.00	23.00
Sodium silicate	14.00	14.00	14.00	14.00
Zeolite	8.20	8.20	8.20	8.20
Chelant (diethylaenetriamine-pentaacetic acid)	0.40	0.40	0.40	0.40
Sodium sulfate	5.50	5.50	5.50	5.50
Water	balance to 100%			

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In Examples 33-34, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Leu 94Gly + Gln200Glu, with substantially similar results.

In Examples 35-36, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Leu 94Gly + Gln200Glu and Gln 57Ser + Leu 94Gly + Gln200Glu, with substantially similar results.

Examples 37-40
Granular Fabric Cleaning Composition

Component	Example No			
	37	38	39	40
Asn74His + Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu	0.10	0.20	0.03	0.05
Val 93Gln + Tyr102Cys + Ser154Glu + Asn198Gln + Thr207Gly	--	--	0.02	0.05
C_{12} alkyl benzene sulfonate	12.00	12.00	12.00	12.00
Zeolite A (1-10 micrometer)	26.00	26.00	26.00	26.00
2-butyl octanoic acid	4.00	4.00	4.00	4.00
C_{12}-C_{14} secondary (2,3) alkyl sulfate, Na salt	5.00	5.00	5.00	5.00
Sodium citrate	5.00	5.00	5.00	5.00
Optical brightener	0.10	0.10	0.10	0.10
Sodium sulfate	17.00	17.00	17.00	17.00
Water and minors	balance to 100%			

In Examples 37-38, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn74His + Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu, with substantially similar results.

In Examples 39-40, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn74His + Gln 57Ser + Asn 60Ser + Leu 94Gly + Gln200Glu and Val 93Gln + Tyr102Cys + Ser154Glu + Asn198Gln + Thr207Gly, with substantially similar results.

Examples 41-42

Granular Fabric Cleaning Composition

Component	Example No.	
	41	42
Linear alkyl benzene sulphonate	11.4	10.70
Tallow alkyl sulphate	1.80	2.40
C_{14-15} alkyl sulphate	3.00	3.10
C_{14-15} alcohol 7 times ethoxylated	4.00	4.00
Tallow alcohol 11 times ethoxylated	1.80	1.80
Dispersant	0.07	0.1
Silicone fluid	0.80	0.80
Trisodium citrate	14.00	15.00
Citric acid	3.00	2.50
Zeolite	32.50	32.10
Maleic acid acrylic acid copolymer	5.00	5.00
Diethylene triamine penta methylene phosphonic acid	1.00	0.20
Ser210Asp	0.30	0.30
Lipase	0.36	0.40
Amylase	0.30	0.30
Sodium silicate	2.00	2.50
Sodium sulphate	3.50	5.20
Polyvinyl pyrrolidone	0.30	0.50
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.1	0.1
Minors	Up to 100	Up to 100

Examples 43-44
Granular Fabric Cleaning Composition

Component	Example No. 43	Example No. 44
Sodium linear C_{12} alkyl benzene-sulfonate	6.5	8.0
Sodium sulfate	15.0	18.0
Zeolite A	26.0	22.0
Sodium nitrilotriacetate	5.0	5.0
Polyvinyl pyrrolidone	0.5	0.7
Tetraacetylethylene diamine	3.0	3.0
Boric acid	4.0	-
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Val199Leu + Ser210Glu	0.4	0.4
Fillers (e.g., silicates; carbonates; perfumes; water)	Up to 100	Up to 100

Example 45
Compact Granular Fabric Cleaning Composition

Component	Weight %
Alkyl Sulphate	8.0
Alkyl Ethoxy Sulphate	2.0
Mixture of C_{25} and C_{45} alcohol 3 and 7 times ethoxylated	6.0
Polyhydroxy fatty acid amide	2.5
Zeolite	17.0
Layered silicate/citrate	16.0
Carbonate	7.0
Maleic acid acrylic acid copolymer	5.0
Soil release polymer	0.4
Carboxymethyl cellulose	0.4
Poly (4-vinylpyridine) -N-oxide	0.1
Copolymer of vinylimidazole and vinylpyrrolidone	0.1
PEG2000	0.2
Asn74Asp + Val197Glu + Gln200Glu + Ser210Glu	0.5
Lipase	0.2
Cellulase	0.2
Tetracetylethylene diamine	6.0
Percarbonate	22.0
Ethylene diamine disuccinic acid	0.3

Suds suppressor	3 5
Disodium-4,4'-bis (2-morpholino -4-anilino-s-triazin-6-ylamino) stilbene-2,2'-disulphonate	0.25
Disodium-4,4'-bis (2-sulfostyril) biphenyl	0.05
Water, Perfume and Minors	Up to 100

Example 46
Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulphonate	7.6
C_{16}-C_{18} alkyl sulfate	1.3
C_{14-15} alcohol 7 times ethoxylated	4.0
Coco-alkyl-dimethyl hydroxyethyl ammonium chloride	1.4
Dispersant	0.07
Silicone fluid	0.8
Trisodium citrate	5.0
Zeolite 4A	15.0
Maleic acid acrylic acid copolymer	4.0
Diethylene triamine penta methylene phosphonic acid	0.4
Perborate	15.0
Tetraacetylethylene diamine	5.0
Smectite clay	10.0
Poly (oxy ethylene) (MW 300,000)	0.3
Tyr208Phe + Leu211Asn	0.4
Lipase	0.2
Amylase	0.3
Cellulase	0.2
Sodium silicate	3 0
Sodium carbonate	10.0
Carboxymethyl cellulose	0 2
Brighteners	0.2
Water, perfume and minors	Up to 100

Example 47
Granular Fabric Cleaning Composition

Component	Weight %
Linear alkyl benzene sulfonate	6.92
Tallow alkyl sulfate	2.05
C_{14-15} alcohol 7 times ethoxylated	4.4
C_{12-15} alkyl ethoxy sulfate - 3 times ethoxylated	0.16
Zeolite	20.2
Citrate	5.5
Carbonate	15.4
Silicate	3.0
Maleic acid acrylic acid copolymer	4.0
Carboxymethyl cellulase	0.31
Soil release polymer	0.30
Asn74His + Val197Glu + Pro204Ala + Ala209Thr + Ser210Glu	0.2
Lipase	0.36
Cellulase	0.13
Perborate tetrahydrate	11.64
Perborate monohydrate	8.7
Tetraacetylethylene diamine	5.0
Diethylene tramine penta methyl phosphonic acid	0.38
Magnesium sulfate	0.40
Brightener	0.19
Perfume, silicone, suds suppressors	0.85
Minors	Up to 100

b.   Liquid fabric cleaning compositions

5   Liquid fabric cleaning compositions of the present invention comprise an effective amount of one or more enzyme variants of the present invention, preferably from about 0.005% to about 5%, more preferably from about 0.01% to about 1%, by weight of active enzyme of the composition. Such liquid fabric cleaning compositions typically additionally comprise an anionic surfactant, a

10   fatty acid, a water-soluble detergency builder and water.

The liquid fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

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Examples 48-52
Liquid Fabric Cleaning Compositions

Component	Example No.				
	48	49	50	51	52
Pro204Ala + Ala209Thr	0.05	0.03	0.30	0.03	0.10
Gln200Glu + Thr207Glu + Ser210Glu	-	-	-	0.01	0.20
C_{12}- C_{14} alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C_{10} alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 48-50 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Pro204Ala + Ala209Thr, with substantially similar results.

In Examples 51-52, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Pro204Ala + Ala209Thr and Gln200Glu + Thr207Glu + Ser210Glu, with substantially similar results.

Examples 53-57
Liquid Fabric Cleaning Compositions

Component	Example No.				
	53	54	55	56	57
Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly	0.05	0.03	0.30	0.03	0.10
Asn74Asp + Ser97Asp + Gln 57Ser + Asn 60Ser	–	–	–	0.01	0.20
C_{12}- C_{14} alkyl sulfate, Na	20.00	20.00	20.00	20.00	20.00
2-butyl octanoic acid	5.00	5.00	5.00	5.00	5.00
Sodium citrate	1.00	1.00	1.00	1.00	1.00
C_{10} alcohol ethoxylate (3)	13.00	13.00	13.00	13.00	13.00
Monethanolamine	2.50	2.50	2.50	2.50	2.50
Water/propylene glycol/ethanol (100:1:1)	balance to 100%				

In Examples 53-55 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly, with substantially similar results.

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In Examples 56-57, any combination of the subtilisin 309 variants recited in Tables 212, among others, are substituted for Tyr102Cys + Ile105Val + Leu124Ile + Ser154Glu + Asn198Gln + Thr207Gly and Asn74Asp + Ser97Asp + Gln 57Ser + Asn 60Ser, with substantially similar results.

Examples 58-59
Granular Fabric Cleaning Composition

Component	Example No.	
	58	59
C_{12-14} alkenyl succinic acid	3.0	8.0
Citric acid monohydrate	10.0	15.0
Sodium C_{12-15} alkyl sulphate	8.0	8.0
Sodium sulfate of C_{12-15} alcohol 2 times ethoxylated	-	3.0
C_{12-15} alcohol 7 times ethoxylated	-	8.0
C_{12-15} alcohol 5 times ethoxylated	8.0	-
Diethylene triamine penta (methylene phosphonic acid)	0.2	-
Oleic acid	1.8	-
Ethanol	4.0	4.0
Propanediol	2.0	2.0
Asn74Asp + Ser210Glu	0.2	0.2
Polyvinyl pyrrolidone	1.0	2.0
Suds suppressor	0.15	0.15
NaOH	up to pH 7.5	
Perborate	0.5	1
Phenol sulphonate	0.1	0.2
Peroxidase	0.4	0.1
Waters and minors	up to 100 parts	

In each of Examples 58 and 59 herein, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn74Asp + Ser210Glu, with substantially similar results.

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Examples 60-62
Liquid Fabric Cleaning Composition

	Component	Example No. 60	61	62
5	Citric Acid	7.10	3.00	3.00
	Fatty Acid	2.00	-	2.00
	Ethanol	1.93	3.20	3.20
	Boric Acid	2.22	3.50	3.50
	Monoethanolamine	0.71	1.09	1.09
10	1,2 Propanediol	7.89	8.00	8.00
	NaCumene Sulfonate	1.80	3.00	3.00
	NaFormate	0.08	0.08	0.08
	NaOH	6.70	3.80	3.80
	Silicon anti-foam agent	1.16	1.18	1.18
15	Ser210Glu	0.0145	-	-
	Asn74Asp + Ser97Asp + Ser210Glu	-	0.0145	-
	Gln200Glu + Ser210Glu	-	-	0.0145
	Lipase	0.200	0.200	0.200
	Cellulase	-	7.50	7.50
20	Soil release polymer	0.29	0.15	0.15
	Anti-foaming agents	0.06	0.085	0.085
	Brightener 36	0.095	-	-
	Brightener 3	-	0.05	0.05
	C_{12} alkyl benzenesulfonic acid	9.86	-	-
25	C_{12-15} alkyl polyethoxylate (2.5) sulfate	13.80	18.00	18.00
	C_{12} glucose amide	-	5.00	5.00
	C_{12-13} alkyl polyethoxylate (9)	2.00	2.00	2.00
	Water, perfume and minors	balance to 100%		

c.   Bar fabric cleaning compositions

30   Bar fabric cleaning compositions of the present invention suitable for hand-washing soiled fabrics contain an effective amount of one or more enzyme variants of the present invention, preferably from about 0.001% to about 10%, more preferably from about 0.01% to about 1% by weight of the composition.

35   The bar fabric cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 63-66
Bar Fabric Cleaning Compositions

Component	Example No.			
	63	64	65	66
Val197Glu	0.3	-	0.1	0.02
Tyr208Phe + Leu211Asn	-	-	0.4	0.03
C_{12}-C_{16} alkyl sulfate, Na	20.0	20.0	20.0	20.00
C_{12}-C_{14} N-methyl glucamide	5.0	5.0	5.0	5.00
C_{11}-C_{13} alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-.10μ)	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
$CaSO_4$	1.0	1.0	1.0	1.00
$MgSO_4$	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

*Can be selected from convenient materials such as $CaCO_3$, talc, clay, silicates, and the like.

In Examples 63-64 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val197Glu, with substantially similar results.

In Examples 65-66, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val197Glu and Tyr208Phe + Leu211Asn, with substantially similar results.

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Examples 67-70

Bar Fabric Cleaning Compositions

Component	Example No.			
	67	68	69	70
Asn 60Ser + Val 93Gln + Gly213Asp	0.3	-	0.1	0.02
Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp	-	0.3	0.4	0.03
C_{12}-C_{16} alkyl sulfate, Na	20.0	20.0	20.0	20.00
C_{12}-C_{14} N-methyl glucamide	5.0	5.0	5.0	5.00
C_{11}-C_{13} alkyl benzene sulfonate, Na	10.0	10.0	10.0	10.00
Sodium carbonate	25.0	25.0	25.0	25.00
Sodium pyrophosphate	7.0	7.0	7.0	7.00
Sodium tripolyphosphate	7.0	7.0	7.0	7.00
Zeolite A (0.1-.10μ)	5.0	5.0	5.0	5.00
Carboxymethylcellulose	0.2	0.2	0.2	0.20
Polyacrylate (MW 1400)	0.2	0.2	0.2	0.20
Coconut monethanolamide	5.0	5.0	5.0	5.00
Brightener, perfume	0.2	0.2	0.2	0.20
$CaSO_4$	1.0	1.0	1.0	1.00
$MgSO_4$	1.0	1.0	1.0	1.00
Water	4.0	4.0	4.0	4.00
Filler*	balance to 100%			

*Can be selected from convenient materials such as $CaCO_3$, talc, clay, silicates, and the like.

In Example 67, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn 60Ser + Val 93Gln + Gly213Asp, with substantially similar results.

In Example 68, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp, with substantially similar results.

In Examples 69-70, any combination of the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Asn 60Ser + Val 93Gln + Gly213Asp and Val 93Gln + Tyr102Cys + Thr207Gly + Gly213Asp, with substantially similar results.

B.   Additional Cleaning Compositions

In addition to the hard surface cleaning, dishwashing and fabric cleaning compositions discussed above, one or more enzyme variants of the present invention may be incorporated into a variety of other cleaning

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compositions where hydrolysis of an insoluble substrate is desired. Such additional cleaning compositions include but are not limited to, oral cleaning compositions, denture cleaning compositions, and contact lens cleaning compositions.

5   1.   Oral cleaning compositions

In another embodiment of the present invention, a pharmaceutically-acceptable amount of one or more enzyme variants of the present invention are included in compositions useful for removing proteinaceous stains from teeth or dentures. As used herein, "oral cleaning compositions" refers to dentifrices, toothpastes, toothgels, toothpowders, mouthwashes, mouth sprays, mouth gels, chewing gums, lozenges, sachets, tablets, biogels, prophylaxis pastes, dental treatment solutions, and the like. Preferably, the oral cleaning compositions comprise from about 0.0001% to about 20% of one or more enzyme variants of the present invention, more preferably from about 0.001% to about 10%, more preferably still from about 0.01% to about 5%, by weight of the composition, and a pharmaceutically-acceptable carrier. As used herein, "pharmaceutically-acceptable" means that drugs, medicaments or inert ingredients which the term describes are suitable for use in contact with the tissues of humans and lower animals without undue toxicity, incompatibility, instability, irritation, allergic response, and the like, commensurate with a reasonable benefit/risk ratio.

Typically, the pharmaceutically-acceptable oral cleaning carrier components of the oral cleaning components of the oral cleaning compositions will generally comprise from about 50% to about 99.99%, preferably from about 65% to about 99.99%, more preferably from about 65% to about 99%, by weight of the composition.

The pharmaceutically-acceptable carrier components and optional components which may be included in the oral cleaning compositions of the present invention are well known to those skilled in the art. A wide variety of composition types, carrier components and optional components useful in the oral cleaning compositions are disclosed in U.S. Patent 5,096,700, Seibel, issued March 17, 1992; U.S. Patent 5,028,414, Sampathkumar, issued July 2, 1991; and U.S. Patent 5,028,415, Benedict, Bush and Sunberg, issued July 2, 1991; all of which are incorporated herein by reference.

The oral cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 71-74
Dentifrice Composition

Component	Example No.			
	71	72	73	74
Val199Leu + Pro204Ala + Thr207Glu +Ser210Glu	2.000	3.500	1.500	2.000
Sorbitol (70% aqueous solution)	35.000	35.000	35.000	35.000
PEG-6*	1.000	1.000	1.000	1.000
Silica dental abrasive**	20.000	20.000	20.000	20.000
Sodium fluoride	0.243	0.243	0.243	0.243
Titanium dioxide	0.500	0.500	0.500	0.500
Sodium saccharin	0.286	0.286	0.286	0.286
Sodium alkyl sulfate (27.9% aqueous solution)	4.000	4.000	4.000	4.000
Flavor	1.040	1.040	1.040	1.040
Carboxyvinyl Polymer***	0.300	0.300	0.300	0.300
Carrageenan****	0.800	0.800	0.800	0.800
Water	balance to 100%			

*PEG-6 = Polyethylene glycol having a molecular weight of 600.

**Precipitated silica identified as Zeodent 119 offered by J.M. Huber.

***Carbopol offered by B.F. Goodrich Chemical Company.

****Iota Carrageenan offered by Hercules Chemical Company.

In Examples 71-74 the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val199Leu + Pro204Ala + Thr207Glu + Ser210Glu, with substantially similar results.

Examples 75-78
Mouthwash Composition

Component	Example No 75	76	77	78
Ser210Asp	3.00	7.50	1.00	5.00
SDA 40 Alcohol	8.00	8.00	8.00	8.00
Flavor	0.08	0.08	0.08	0.08
Emulsifier	0.08	0.08	0.08	0.08
Sodium Fluoride	0.05	0.05	0.05	0.05
Glycerin	10.00	10.00	10.00	10.00
Sweetener	0.02	0.02	0.02	0.02
Benzoic acid	0.05	0.05	0.05	0.05
Sodium hydroxide	0.20	0.20	0.20	0.20
Dye	0.04	0.04	0.04	0.04
Water	balance to 100%			

In Examples 75-78, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser210Asp, with substantially similar results.

Examples 79-82
Lozenge Composition

Component	Example No. 79	80	81	82
Tyr208Phe + Leu211Asn	0.01	0.03	0.10	0.02
Sorbitol	17.50	17.50	17.50	17.50
Mannitol	17.50	17.50	17.50	17.50
Starch	13.60	13.60	13.60	13.60
Sweetener	1.20	1.20	1.20	1.20
Flavor	11.70	11.70	11.70	11.70
Color	0.10	0.10	0.10	0.10
Corn Syrup	balance to 100%			

In Examples 79-82, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Tyr208Phe + Leu211Asn, with substantially similar results.

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Examples 83-86
Chewing Gum Composition

Component	Example No.			
	83	84	85	86
Val199Met + Pro204Ala + Thr207Glu	0.03	0.02	0.10	0.05
Sorbitol crystals	38.44	38.40	38.40	38.40
Paloja-T gum base*	20.00	20.00	20.00	20.00
Sorbitol (70% aqueous solution)	22.00	22.00	22.00	22.00
Mannitol	10.00	10.00	10.00	10.00
Glycerine	7.56	7.56	7.56	7.56
Flavor	1.00	1.00	1.00	1.00

*Supplied by L.A. Dreyfus Company.

In Examples 83-86, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Val199Met + Pro204Ala + Thr207Glu, with substantially similar results.

2.   Denture cleaning compositions

In another embodiment of the present invention, denture cleaning compositions for cleaning dentures outside of the oral cavity comprise one or more enzyme variants of the present invention.   Such denture cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.0001% to about 50% of one or more of the enzyme variants, more preferably from about 0.001% to about 35%, more preferably still from about 0.01% to about 20%, by weight of the composition, and a denture cleansing carrier.   Various denture cleansing composition formats such as effervescent tablets and the like are well known in the art (see for example U.S. Patent 5,055,305, Young, incorporated herein by reference), and are generally appropriate for incorporation of one or more of the enzyme variants for removing proteinaceous stains from dentures.

The denture cleaning composition embodiment of the present invention is illustrated by the following examples.

Examples 87-90

Two-layer Effervescent Denture Cleansing Tablet

Component	Example No 87	88	89	90
Acidic Layer				
Ser210Glu	1.0	1.5	0.01	0.05
Tartaric acid	24.0	24.0	24.00	24.00
Sodium carbonate	4.0	4.0	4.00	4.00
Sulphamic acid	10.0	10.0	10.00	10.00
PEG 20,000	4.0	4.0	4.00	4.00
Sodium bicarbonate	24.5	24.5	24.50	24.50
Potassium persulfate	15.0	15.0	15.00	15.00
Sodium acid pyrophosphate	7.0	7.0	7.00	7.00
Pyrogenic silica	2.0	2.0	2.00	2.00
TAED*	7.0	7.0	7.00	7.00
Ricinoleylsulfosuccinate	0.5	0.5	0.50	0.50
Flavor	1.0	1.0	1.00	1.00
Alkaline Layer				
Sodium perborate monohydrate	32.0	32.0	32.00	32.00
Sodium bicarbonate	19.0	19.0	19.00	19.00
EDTA	3.0	3.0	3.00	3.00
Sodium tripolyphosphate	12.0	12.0	12.00	12.00
PEG 20,000	2.0	2.0	2.00	2.00
Potassium persulfate	26.0	26.0	26.00	26.00
Sodium carbonate	2.0	2.0	2.00	2.00
Pyrogenic silica	2.0	2.0	2.00	2.00
Dye/flavor	2.0	2.0	2.00	2.00

*Tetraacetylethylene diamine

In Examples 87-90, the subtilisin 309 variants recited in Tables 3-38, among others, are substituted for Ser210Glu, with substantially similar results.

3.   Contact Lens Cleaning Compositions

In another embodiment of the present invention, contact lens cleaning compositions comprise one or more enzyme variants of the present invention. Such contact lens cleaning compositions comprise an effective amount of one or more of the enzyme variants, preferably from about 0.01% to about 50% of one or more of the enzyme variants, more preferably from about 0.01% to about 20%, more preferably still from about 1% to about 5%, by weight of the composition, and a contact lens cleaning carrier.   Various contact lens

cleaning composition formats such as tablets, liquids and the like are well
known in the art (see for example U.S. Patent 4,863,627, Davies, Meaken and
Rees, issued September 5, 1989; U.S. Patent Re. 32,672, Huth, Lam and
Kirai, reissued May 24, 1988; U.S. Patent 4,609,493, Schäfer, issued
September 2, 1986; U.S. Patent, 4,690,793, Ogunbiyi and Smith, issued
September 1, 1987; U.S. Patent 4,614,549, Ogunbiyi, Riedhammer and Smith,
issued September 30, 1986; and U.S. Patent 4,285,738, Ogata, issued August
25, 1981; each of which are incorporated herein by reference), and are
generally appropriate for incorporation of one or more enzyme variants of the
present invention for removing proteinaceous stains from contact lens.

The contact lens cleaning composition embodiment of the present
invention is illustrated by the following examples.

Examples 91-94
Enzymatic Contact Lens Cleaning Solution

Component	Example No.			
	91	92	93	94
Val199Leu + Ser210Asp	0.01	0.5	0.1	2.0
Glucose	50.00	50.0	50.0	50.0
Nonionic surfactant (polyoxyethlene-polyoxypropylene copolymer)	2.00	2.0	2.0	2.0
Anionic surfactant (polyoxyethylene-alkylphenylether sodium sulfricester)	1.00	1.0	1.0	1.0
Sodium chloride	1.00	1.0	1.0	1.0
Borax	0.30	0.3	0.3	0.3
Water	balance to 100%			

In Examples 91-94, the subtilisin 309 variants recited in Tables 3-38,
among others, are substituted for Val199Leu + Ser210Asp, with substantially
similar results.

While particular embodiments of the subject invention have been
described, it will be obvious to those skilled in the art that various changes and
modifications of the subject invention can be made without departing from the
spirit and scope of the invention. It is intended to cover, in the appended
claims, all such modifications that are within the scope of the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANT: PHILIP F. BRODE, III
                      BOBBY L. BARNETT
                      DONN N. RUBINGH
                      CHANCHAL K. GHOSH

    (ii) TITLE OF INVENTION: SUBTILISIN 309 VARIANTS WITH
                                  DECREASED ADSORPTION AND
                                  INCREASED HYDROLYSIS

    (iii) NUMBER OF SEQUENCES: 2

    (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: THE PROCTER & GAMBLE COMPANY
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        (C) CITY: ROSS
        (D) STATE: OH
        (E) COUNTRY: USA
        (F) ZIP: 45061

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: Floppy disk
        (B) COMPUTER: IBM PC compatible
        (C) OPERATING SYSTEM: PC-DOS/MS-DOS
        (D) SOFTWARE: PatentIn Release #1.0, Version #1.25

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

    (viii) ATTORNEY/AGENT INFORMATION:
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        (B) REGISTRATION NUMBER: 34,804
        (C) ATTORNEY DOCKET NO.: 5233R

    (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: 513-627-2858
        (B) TELEFAX: 513-627-0260

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 269 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Gln Ser Val Pro Trp Gly Ile Ser Arg Val Gln Ala Pro Ala Ala
1   5   10   15

His Asn Arg Gly Leu Thr Gly Ser Gly Val Lys Val Ala Val Leu Asp
      20   25   30

Thr Gly Ile Ser Thr His Pro Asp Leu Asn Ile Arg Gly Gly Ala Ser
      35   40   45

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        Phe Val Pro Gly Glu Pro Ser Thr Gln Asp Gly Asn Gly His Gly Thr
              50              55                  60

        His Val Ala Gly Thr Ile Ala Ala Leu Asn Asn Ser Ile Gly Val Leu
        65                  70                  75                  80

        Gly Val Ala Pro Ser Ala Glu Leu Tyr Ala Val Lys Val Leu Gly Ala
                          85                  90                  95

        Ser Gly Ser Gly Ser Val Ser Ser Ile Ala Gln Gly Leu Glu Trp Ala
                          100                 105                 110

        Gly Asn Asn Gly Met His Val Ala Asn Leu Ser Leu Gly Ser Pro Ser
                          115                 120                 125

        Pro Ser Ala Thr Leu Glu Gln Ala Val Asn Ser Ala Thr Ser Arg Gly
                130                 135                 140

        Val Leu Val Val Ala Ala Ser Gly Asn Ser Gly Ala Gly Ser Ile Ser
        145                 150                 155                 160

        Tyr Pro Ala Arg Tyr Ala Asn Ala Met Ala Val Gly Ala Thr Asp Gln
                          165                 170                 175

        Asn Asn Asn Arg Ala Ser Phe Ser Gln Tyr Gly Ala Gly Leu Asp Ile
                          180                 185                 190

        Val Ala Pro Gly Val Asn Val Gln Ser Thr Tyr Pro Gly Ser Thr Tyr
                          195                 200                 205

        Ala Ser Leu Asn Gly Thr Ser Met Ala Thr Pro His Val Ala Gly Ala
                          210                 215                 220

        Ala Ala Leu Val Lys Gln Lys Asn Pro Ser Trp Ser Asn Val Gln Ile
        225                 230                 235                 240

        Arg Asn His Leu Lys Asn Thr Ala Thr Ser Leu Gly Ser Thr Asn Leu
                          245                 250                 255

        Tyr Gly Ser Gly Leu Val Asn Ala Glu Ala Ala Thr Arg
                          260                 265
```

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 275 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
     Ala Gin Ser Val Pro Tyr Gly Val Ser Gln Ile Lys Ala Pro Ala Leu
     1               5                   10                  15

     His Ser Gln Gly Tyr Thr Gly Ser Asn Val Lys Val Ala Val Ile Asp
                     20                  25                  30

     Ser Gly Ile Asp Ser Ser His Pro Asp Leu Lys Val Ala Gly Gly Ala
                 35                  40                  45

     Ser Met Val Pro Ser Glu Thr Asn Pro Phe Gln Asp Asn Asn Ser His
         50                  55                  60

     Gly Thr His Val Ala Gly Thr Val Ala Ala Leu Asn Asn Ser Ile Gly
     65                  70                  75                  80

     Val Leu Gly Val Ala Pro Ser Ala Ser Leu Tyr Ala Val Lys Val Leu
                     85                  90                  95

     Gly Ala Asp Gly Ser Gly Gln Tyr Ser Trp Ile Ile Asn Gly Ile Glu
                 100                 105                 110

     Trp Ala Ile Ala Asn Asn Met Asp Val Ile Asn Met Ser Leu Gly Gly
                 115                 120                 125

     Pro Ser Gly Ser Ala Ala Leu Lys Ala Ala Val Asp Lys Ala Val Ala
         130                 135                 140

     Ser Gly Val Val Val Val Ala Ala Ala Gly Asn Glu Gly Thr Ser Gly
     145                 150                 155                 160

     Ser Ser Ser Thr Val Gly Tyr Pro Gly Lys Tyr Pro Ser Val Ile Ala
                     165                 170                 175

     Val Gly Ala Val Asp Ser Ser Asn Gln Arg Ala Ser Phe Ser Ser Val
                 180                 185                 190

     Gly Pro Glu Leu Asp Val Met Ala Pro Gly Val Ser Ile Gln Ser Thr
                 195                 200                 205

     Leu Pro Gly Asn Lys Tyr Gly Ala Tyr Asn Gly Thr Ser Met Ala Ser
         210                 215                 220

     Pro His Val Ala Gly Ala Ala Ala Leu Ile Leu Ser Lys His Pro Asn
     225                 230                 235                 240

     Trp Thr Asn Thr Gln Val Arg Ser Ser Leu Glu Asn Thr Thr Thr Lys
                     245                 250                 255

     Leu Gly Asp Ser Phe Tyr Tyr Gly Lys Gly Leu Ile Asn Val Gln Ala
                 260                 265                 270

     Ala Ala Gln
         275
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What is claimed is

1.   A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, characterized in that the modified amino acid sequence comprises a substitution at one or more of positions 193, 194, 195, 196, 197, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214, wherein

    a. when a substitution occurs at position 193, the substituting amino acid is Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    b. when a substitution occurs at position 194, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    c. when a substitution occurs at position 195, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;

    d. when a substitution occurs at position 196, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

    e. when a substitution occurs at position 197, the substituting amino acid is Met, Cys, Ala, His, Pro, Gly, Gln, Ser, Asp or Glu;

    f. when a substitution occurs at position 199, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    g. when a substitution occurs at position 200, the substituting amino acid is 200 Asn or Ser;

    h. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;

    i. when a substitution occurs at position 202, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    j. when a substitution occurs at position 203, the substituting amino acid is Ile, Met, Ala, His, Pro, Gln, Asn, Ser, or Asp;

    k. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;

    l. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

    m. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;

    n. when a substitution occurs at position 207, the substituting amino acid is Pro, Gly, Gln, Asn, Ser or Glu;

    o. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;

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    p. when a substitution occurs at position 209, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    q. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;

    r. when a substitution occurs at position 211, the substituting amino acid is Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, or Asp;

    s. when a substitution occurs at position 212, the substituting amino acid is Glu;

    t. when a substitution occurs at position 213, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and

    u. when a substitution occurs at position 214, the substituting amino acid is Pro, Gly, Gln, Asn, Asp, Ser or Glu;

whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309; preferably

    i.   when a substitution occurs at position 200, the substituting amino acid is Ser;

    ii.   when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

    iii.   when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;

    iv.   when a substitution occurs at position 209, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and

    v.   when a subtilisin occurs at position 210, the substituting amino acid is Asp or Glu;

more preferably when a substitution occurs at one or more of positions 193, 194, 195, 196, 197, 199, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, or 214, the substituting amino acid for any of positions 193, 194, 195, 196, 197, 199, 201, 202, 204, 205, 206, 208, 209, 210, 211, 212, 213, or 214 is Asp or Glu; when a substitution occurs at position 203, the substituting amino acid for position 203 is Asp; and when a substitution occurs at one or more positions 207 or 212, the substituting amino acid for either of positions 207 or 212 is Glu.

    2.   The subtilisin 309 variant of Claim 1 having a single amino acid substitution wherein the substitution is:

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    a.   Glu for Thr at position 207,

    b.   Glu for Ser at position 210, or

    c.   Asp for Ser at position 210.

    3.   A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, characterized in that the modified amino acid sequence comprises a substitution at three or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 is substituted, wherein

    a. when a substitution occurs at position 193, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    b. when a substitution occurs at position 194, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    c. when a substitution occurs at position 195, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;

    d. when a substitution occurs at position 196, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

    e. when a substitution occurs at position 197, the substituting amino acid is Met, Cys, Ala, His, Pro, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    f. when a substitution occurs at position 198, the substituting amino acid is Glu, Gln, Asp or Ser;

    g. when a substitution occurs at position 199, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    h. when a substitution occurs at position 200, the substituting amino acid is Asn, Ser Glu, or Asp;

    i. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;

    j. when a substitution occurs at position 202, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    k. when a substitution occurs at position 203, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    l. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;

    m. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

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    n. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;

    o. when a substitution occurs at position 207, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    p. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Thr, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;

    q. when a substitution occurs at position 209, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

    r. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;

    s. when a substitution occurs at position 211, the substituting amino acid is Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Glu, or Asp;

    t. when a substitution occurs at position 212, the substituting amino acid is Gln, Ser, Asp, or Glu;

    u. when a substitution occurs at position 213, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and

    v. when a substitution occurs at position 214, the substituting amino acid is Pro, Gly, Gln, Asn, Asp, Ser or Glu;

whereby the 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309; preferably

    i. when a substitution occurs at position 200, the substituting amino acid is Asn, Glu, Asp, or Ser;

    ii. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;

    iii. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

    iv. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;

    v. when a substitution occurs at position 209, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and

    vi. when a substitution occurs at position 210, the substituting amino acid is Glu or Asp .

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    4.   The subtilisin 309 variant of Claim 3 comprising two amino acid substitutions, wherein the substitutions are:

        a.   Glu for Thr at position 207 and Glu for Ser at position 210;

        b.   Leu for Val at position 197 and Glu for Ser at position 210; or

        c.   Leu for Val at position 199 and Asp for Ser at position 210.

        d.   Ala for Pro at position 204 and Thr for Ala at position 209;

        e.   Phe for Tyr at position 208 and Asn for Leu at position 211;

        f.   Asp for Asn at position 74 and Glu for Ser at position 210; or

        g.   Glu for Lys at position 207 and Glu for Ala at position 210

    5.   A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, characterized in that the modified amino acid sequence comprises a substitution at three or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214 is substituted, wherein

        a. when a substitution occurs at position 193, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

        b. when a substitution occurs at position 194, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

        c. when a substitution occurs at position 195, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;

        d. when a substitution occurs at position 196, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

        e. when a substitution occurs at position 197, the substituting amino acid is Met, Cys, Ala, His, Pro, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

        f. when a substitution occurs at position 198, the substituting amino acid is Glu, Gln, Asp or Ser;

        g. when a substitution occurs at position 199, the substituting amino acid is Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

        h. when a substitution occurs at position 200, the substituting amino acid is Asn, Ser Glu, or Asp;

        i. when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;

        j. when a substitution occurs at position 202, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;

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k. when a substitution occurs at position 203, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

l. when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;

m. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

n. when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;

o. when a substitution occurs at position 207, the substituting amino acid is Pro, Gly, Gln, Asn, Ser, Asp or Glu;

p. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Thr, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;

q. when a substitution occurs at position 209, the substituting amino acid is His, Thr, Pro, Gly, Gln, Asn, Ser, Asp or Glu;

r. when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;

s. when a substitution occurs at position 211, the substituting amino acid is Ile, Val, Met, Cys, Ala, His, Thr, Pro, Gly, Gln, Asn, Ser, Glu, or Asp;

t. when a substitution occurs at position 212, the substituting amino acid is Gln, Ser, Asp, or Glu;

u. when a substitution occurs at position 213, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and

v. when a substitution occurs at position 214, the substituting amino acid is Pro, Gly, Gln, Asn, Asp, Ser or Glu;

whereby the 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309; preferably

i.   when a substitution occurs at position 200, the substituting amino acid is Asn, Glu, Asp, or Ser;

ii.   when a substitution occurs at position 204, the substituting amino acid is Gly, Gln, Asn, Ser, Asp or Glu;

iii. when a substitution occurs at position 205, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu;

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  iv. when a substitution occurs at position 208, the substituting amino acid is Leu, Ile, Val, Met, Cys, Ala, His, Pro, Gly, Gln, Asn, Asp or Glu;

  v. when a substitution occurs at position 209, the substituting amino acid is Pro, Gln, Asn, Ser, Asp or Glu; and

  vi. when a substitution occurs at position 210, the substituting amino acid is Glu or Asp .

  6. The subtilisin 309 variant of Claim 5 comprising three amino acid substitutions, wherein the substitutions are:

  a. Glu substituted for Gln at position 200, Ala substituted for Gly at position 205, and Glu substituted for Ser at position 210;

  b. Leu substituted for Val at position 199, Ala substituted for Pro at position 204, and Glu substituted for Thr at position 207;

  c. Asp for Asn at position 74, Glu substituted for Gln at position 200, and Glu substituted for Ser at position 210; or

  d. Glu for Gln at position 200, Glu for Thr at position 207, and Glu for Ser at position 210.

  7. The subtilisin 309 variant of Claim 5 having the following four substitutions: Ala substituted for Pro at position 204, Glu substituted for Thr at position 207, Glu substituted for Ser at position 210, and Asp substituted for Asn at position 74.

  8. The subtilisin 309 variant of Claim 5 having the following five substitutions: Leu for Ile at position 199, Ala for Pro at position 204, Glu for Lys at position 207, Glu for Ala at positions 210, and Asp substituted for Asn at position 74.

  9. A subtilisin 309 variant having a modified amino acid sequence of subtilisin 309 wild-type amino acid sequence, the wild-type amino acid sequence comprising a first loop region, a second loop region, a third loop region, a fourth loop region and a fifth loop region; characterized in that the modified amino acid sequence comprises a substitution at one or more positions in one or more of the loop regions; wherein

  A. when a substitution occurs in the first loop region, the substitution occurs at one or more of positions 57, 58, 59, 60, 61, 63, or 64; wherein

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    a.   when a substitution occurs at position 57, the substituting amino acid is Asn, Asp, Glu or Ser;

    b.   when a substitution occurs at position 58, the substituting amino acid is Glu;

    c.   when a substitution occurs at position 59, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    d.   when a substitution occurs at position 60, the substituting amino acid is Asp, Gln, Glu or Ser;

    e.   when a substitution occurs at position 61, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    f.   when a substitution occurs at position 63, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and

    g.   when a substitution occurs at position 64, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;

B.   when a substitution occurs in the second loop region, the substitution occurs at one or more of positions 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104 or 105; wherein

    a.   when a substitution occurs at position 93, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;

    b.   when a substitution occurs at position 94, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;

    c.   when a substitution occurs at position 95, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    d.   when a substitution occurs at position 96, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;

    e.   when a substitution occurs at position 97, the substituting amino acid is Asp or Glu;

    f.   when a substitution occurs at position 98, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    g.   when a substitution occurs at position 99, the substituting amino acid is Asp or Glu;

    h.   when a substitution occurs at position 100, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    i.   when a substitution occurs at position 101, the substituting amino acid is Asp or Glu;

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j.   when a substitution occurs at position 102, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;

k.   when a substitution occurs at position 103, the substituting amino acid is Asp or Glu;

l.   when a substitution occurs at position 104, the substituting amino acid is Asp or Glu;

m.   when a substitution occurs at position 105, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val; and

C.   when a substitution occurs in the third loop region, the substitution occurs at one or more of positions 124, 125, 126, 127, 128, 129, 130 or 131; wherein

    a.   when a substitution occurs at position 124, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Ser, Thr or Val;

    b.   when a substitution occurs at position 125, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    c.   when a substitution occurs at position 126, the substituting amino acid is Asp or Glu;

    d.   when a substitution occurs at position 127, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;

    e.   when a substitution occurs at position 128, the substituting amino acid is Asp or Glu;

    f.   when a substitution occurs at position 129, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;

    g.   when a substitution occurs at position 130, the substituting amino acid is Asp or Glu; and

    h.   when a substitution occurs at position 131, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser, Thr;

D.   when a substitution occurs in the fourth loop region, the substitution occurs at one or more of positions 152, 153, 154, 155, 156, 157, 158, 159, 160 or 161; wherein

    a.   when a substitution occurs at position 152, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    b.   when a substitution occurs at position 153, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    c.   when a substitution occurs at position 154, the substituting amino acid is Asp or Glu;

    d.   when a substitution occurs at position 155, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    e.   when a substitution occurs at position 156, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;

    f.   when a substitution occurs at position 157, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

    g.   when a substitution occurs at position 158, the substituting amino acid is Asp or Glu;

    h.   when a substitution occurs at position 159, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Leu, Met, Pro, Ser, Thr or Val;

    i.   when a substitution occurs at position 160, the substituting amino acid is Asp or Glu; and

    j.   when a substitution occurs at position 161, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val;

E.   when a substitution occurs in the fifth loop region, the substitution occurs at one or more of positions 181, 182, 183, 184 or 185; wherein

    a.   when a substitution occurs at position 181, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;

    b.   when a substitution occurs at position 182, the substituting amino acid is Asp or Glu;

    c.   when a substitution occurs at position 183, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr, Tyr or Val;

    d.   when a substitution occurs at position 184, the substituting amino acid is Asp or Glu; and

    e.   when a substitution occurs at position 185, the substituting amino acid is Asn, Asp, Glu or Ser;

whereby the subtilisin 309 variant has decreased adsorption to, and increased hydrolysis of, an insoluble substrate as compared to wild-type subtilisin 309.

10.   The subtilisin 309 variant of Claim 9, wherein the subtilisin 309 wild-type amino acid sequence further comprises a sixth loop region, wherein the modified amino acid sequence further comprises one or more substitutions

in the sixth loop region; wherein the substitution(s) in the sixth loop region occurs at one or more of positions 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213 or 214; wherein

a.   when a substitution occurs at position 193, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;

b.   when a substitution occurs at position 194, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;

c.   when a substitution occurs at position 195, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;

d.   when a substitution occurs at position 196, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

e.   when a substitution occurs at position 197, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;

f.   when a substitution occurs at position 198, the substituting amino acid is Asp, Gln, Glu or Ser;

g.   when a substitution occurs at position 199, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Met, Pro, Ser or Thr;

h.   when a substitution occurs at position 200, the substituting amino acid is Asn, Asp, Glu or Ser;

i.   when a substitution occurs at position 201, the substituting amino acid is Asp or Glu;

j.   when a substitution occurs at position 202, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;

k.   when a substitution occurs at position 203, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val;

l.   when a substitution occurs at position 204, the substituting amino acid is Asn, Asp, Gln, Glu, Gly or Ser;

m.   when a substitution occurs at position 205, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser;

n.   when a substitution occurs at position 206, the substituting amino acid is Asp or Glu;

o.   when a substitution occurs at position 207, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser;

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    p.   when a substitution occurs at position 208, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Pro, Ser, Thr or Val;

    q.   when a substitution occurs at position 209, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, His, Pro, Ser or Thr;

    r.   when a substitution occurs at position 210, the substituting amino acid is Asp or Glu;

    s.   when a substitution occurs at position 211, the substituting amino acid is Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Met, Pro, Ser, Thr or Val;

    t.   when a substitution occurs at position 212, the substituting amino acid is Asp, Gln, Glu or Ser;

    u.   when a substitution occurs at position 213, the substituting amino acid is Asn, Asp, Gln, Glu, Pro or Ser; and

    v.   when a substitution occurs at position 214, the substituting amino acid is Asn, Asp, Gln, Glu, Gly, Pro or Ser.

11.   A cleaning composition selected from the group consisting of a hard surface cleaning composition, a dishwashing composition, an oral cleaning composition, a denture cleansing composition, a contact lens cleaning composition and a fabric cleaning composition, characterized in that the cleaning composition comprises the subtilisin 309 variant of any of Claims 1-11 and a cleaning composition carrier.

12.   The subtilisin 309 variant of any of Claims 1-11 wherein the modified amino acid sequence further comprises His or Asp substituted for Asn at position 74.

13.   The cleaning composition of Claim 11, wherein the cleaning composition is a hard surface cleaning composition.

14.   The cleaning composition of Claim 11; wherein the cleaning composition is a fabric cleaning composition; preferably the composition is in the form of a liquid.

15.   A DNA sequence 309 gene encoding the subtilisin 309 variant of any of Claims 1-11.

Comparison of subtilisin sequences from:

B.amyloliquefaciens

B.lentus

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                 10         20         30
01 A Q S V P Y G V S Q I K A P A L H S Q G Y T G S N V K V A V I D S G I D S S H P

   A Q S V P W G I S R V Q A P A A H N R G L T G S G V K V A V L D T G I S T * H P

              50         60         70
41 D L K V A G G A S M V P S E T N P F Q D N N S H G T H V A G T V A A L N N S I G

   D L N I R G G A S F V P G E * P S T Q D G N G H G T H V A G T I A A L N N S I G

              90        100        110
81 V L G V A P S A S L Y A V K V L G A D G S G Q Y S W I I N G I E W A I A N N M D

   V L G V A P S A E L Y A V K V L G A S G S G S V S S I A Q G L E W A G N N G M H

              130        140        150
121 V I N M S L G G P S G S A A L K A A V D K A V A S G V V V V A A A G N E G T S G

   V A N L S L G S P S P S A T L E Q A V N S A T S R G V L V V A A S G N S G A G S
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FIG 1/A

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161                    170                    180                    190
S S S T V G Y P G K Y P S V I A V G A V D S S N Q R A S F S S V G P E L D V M A

* * * * I S Y P A R Y A N A H A V G A T D Q N N N R A S F S Q Y G A G L D I V A

201                    210                    220                    230
P G V S I Q S T L P G N K Y G A Y N G T S M A S P H V A G A A A L I L S K H P N

P G V N V Q S T Y P G S T Y A S L H G T S M A T P H V A G A A A L V K Q K N P S

241                    250                    260                    270
W T N T Q V R S S L E N T T T K L G D S P Y Y G K G L I N V Q A A A Q

W S N V Q I R N H L K N T A T S L G S T N L Y G S G L V N A E A A T R
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FIG 1/B